

Minireviews

Cancer and the Dopamine D₂ Receptor: A Pharmacological Perspective

Jillian S. Weissenrieder, Jeffrey D. Neighbors, Richard B. Mailman, and Raymond J. Hohl

*Biomedical Sciences Program (J.S.W.) and Departments of Medicine (J.D.N., R.J.H.) and Pharmacology (J.D.N., R.B.M., R.J.H.),
Penn State College of Medicine and Penn State Cancer Institute, Hershey, Pennsylvania*

Received January 25, 2019; accepted April 16, 2019

ABSTRACT

The dopamine D₂ receptor (D₂R) family is upregulated in many cancers and tied to stemness. Reduced cancer risk has been correlated with disorders such as schizophrenia and Parkinson's disease, in which dopaminergic drugs are used. D₂R antagonists are reported to have anticancer efficacy in cell culture and animal models where they have reduced tumor growth, induced autophagy, affected lipid metabolism, and caused apoptosis, among other effects. This has led to several hypotheses, the most prevalent being that D₂R ligands may be a novel approach to cancer chemotherapy. This hypothesis is appealing because

of the large number of approved and experimental drugs of this class that could be repurposed. We review the current state of the literature and the evidence for and against this hypothesis. When the existing literature is evaluated from a pharmacological context, one of the striking findings is that the concentrations needed for cytotoxic effects of D₂R antagonists are orders of magnitude higher than their affinity for this receptor. Although additional definitive studies will provide further clarity, our hypothesis is that targeting D₂-like dopamine receptors may only yield useful ligands for cancer chemotherapy in rare cases.

The Concatenation of Cancer and Neuropharmacology

The serendipitous discovery of chlorpromazine (Delay et al., 1952; Delay and Deniker, 1955) over 60 years ago may be considered a landmark in several ways. Besides offering the first effective treatment of some of the symptoms of schizophrenia, it opened new doors to an understanding of the chemoarchitecture of the brain, especially the role of dopamine (Carlsson et al., 1958; Carlsson and Lindqvist, 1963).

This work was supported by Public Health grants, the Penn State Cancer Institute, the Pritchard Distinguished Graduate Fellowship (Penn State College of Medicine, Hershey, PA), and the National Institutes of Health National Cancer Institute [2T32CA060395-21A1].
<https://doi.org/10.1124/jpet.119.256818>.

This led to millions of people being treated with drugs that targeted dopamine receptors. In psychiatry, this complicated a decades-long debate about whether schizophrenia itself affected cancer risk. For a review, see Gulbinat et al. (1992), who noted that pharmacological mechanisms were of particular interest, especially because some phenothiazine-based drugs had antitumor activity in murine leukemia and melanoma, and high concentrations of the antipsychotics or their metabolites were found in the lung (Driscoll et al., 1978). These latter findings might explain a lower occurrence of malignancies sometimes reported in schizophrenics. Conversely, because classic antipsychotics markedly increased serum prolactin resulting from antagonism of inhibitory dopamine receptors on anterior pituitary lactotrophs, this also might explain an increased risk of breast cancer in females

ABBREVIATIONS: Akt, protein kinase B; AML, acute myeloid leukemia; AraC, Cytosine arabinoside; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N,N-tetraacetic acid acetoxymethyl ester; CHO, Chinese hamster ovary; CSCs, cancer stem cells; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinases; FOX, Forkhead box; GFP, green fluorescent protein; GBM, glioblastoma; 10 GPCR, G protein-coupled receptor; Go6976, 5,6,7,13-Tetrahydro-13-methyl-5-oxo-12H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-12-propanenitrile; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; L-741,626, 3-[4-(4-chlorophenyl)-4-hydroxypiperidinyl]methylindole; LXR, liver X receptors; MAPK, mitogen-activated protein kinase; NGB2904, N-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyl]-9H-fluorene-2-carboxamide; ONC201, 7-benzyl-4-(2-methylbenzyl)-1,2,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(4H)-one; PD, Parkinson disease; PG01037, N-[(2E)-4-[4-(2,3-dichlorophenyl)-1-piperazinyl]-2-buten-1-yl]-4-(2-pyridyl)-benzamide; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PNU 96415E, 1-[2-(3,4-dihydro-1H-2-benzopyran-1-yl)ethyl]-4-(4-fluorophenyl)piperazine; PPAR, Peroxisome proliferator-activated receptor; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; shRNA, short-hairpin RNA; siRNA, short-interfering RNA; SCZ, reactive oxygen species; SB-277011A, N-{trans-4-[2-(6-cyano-3,4-dihydroisoquinolin-2(1H)-yl)ethyl]cyclohexyl}quinoline-4-carboxamide; ROS, Schizophrenia; SKF-83,959, 6-Chloro-2,3,4,5-tetrahydro-3-methyl-1-(3-methylphenyl)-1H-3-benzazepine-7,8-diol; SREBP, sterol regulatory element-binding proteins; STAT, signal transducer and activator of transcription; TRAIL, TNF-related apoptosis-inducing ligand; U99194, 2,3-dihydro-5,6-dimethoxy-N, N-dipropyl-1H-inden-2-amine; VEGFR, vascular EGFR.

(Gulbinat et al., 1992). These early observations led to the hypotheses, first suggested in 1972, that dopamine agonists (then all of the D₂ type) might be a potential therapeutic approach in cancer (Csatary, 1972), as will be discussed later.

Dopamine Receptors

Dopamine receptors are members of the heptahelical G protein-coupled receptor (GPCR) superfamily and are divided pharmacologically into two subfamilies (Fig. 1): “D₁-like” and “D₂-like” (Garau et al., 1978; Kebabian and Calne, 1979). The molecular biology and pharmacology of these receptors have been the subject of numerous reviews and books (Neve and Neve, 1997; Mailman and Huang, 2007). Dopamine receptors are encoded by five genes, with *DRD1* and *DRD5* encoding the two D₁-like receptors (D₁ and D₅), and *DRD2*, *DRD3*, and *DRD4* encoding four expressed mammalian proteins (D_{2long}, D_{2short}, D₃, and D₄). D_{2long} and D_{2short} are splice variants from *DRD2* and together are the most highly expressed of the D₂-like receptors (Dal Toso et al., 1989; Giros et al., 1989; Monsma et al., 1989b; Chio et al., 1990). As noted earlier, the first drugs that were shown to bind to dopamine receptors (e.g., chlorpromazine) were discovered serendipitously because of effects in controlling positive symptoms of schizophrenia. The target of early antipsychotic drugs was soon identified, then validated, via radioreceptor studies and receptor cloning (Burt et al., 1976; Seeman et al., 1976; Dal Toso et al., 1989; Giros et al., 1989; Monsma et al., 1989a, 1990). When using drugs as research tools, it is imperative to understand the relative effects of a molecule on both primary and secondary targets; antipsychotics in particular have many off-target actions. In addition, although they may have selectivity for one subfamily of dopamine receptor, there is often much less selectivity for an individual member (e.g., D₂ vs. D₃ vs. D₄). Thus, when we discuss clinical findings, reference to “D₂” will be a reference to D₂-like affinity unless otherwise specified.

There is a rich literature on both agonist and antagonist effects on dopamine receptors, but it has largely been focused on central nervous system modulation of dopamine function in the context of schizophrenia and other brain disorders

(Neve and Neve, 1997). On the periphery, dopamine is known to play an important role in cardiovascular control and kidney function. The notion that dopamine receptor ligands might affect the biology of neoplastic cells independent of their actions on neurotransmission is provocative, and offers both a novel mechanism and the ability to both purpose and repurpose the huge libraries of dopaminergic ligands and drugs that have resulted from neuropharmacological drug discovery and development (Schalop and Allen, 2016). Thus, an examination of this arena is timely.

Clinical Studies of Dopaminergic Drugs and Cancer

Correlative Studies and Case Reports Support a Role for the D₂ Receptor in Cancer Development and Treatment Response. To date, all antipsychotic drugs engage D₂ receptors, usually as antagonists (Creese et al., 1976; Mailman, 2007; Boyd and Mailman, 2012), whereas therapy for Parkinson disease (PD) relies primarily on activation of dopamine receptors indirectly via levodopa, or directly by direct agonists (Mailman and Huang, 2007). The accepted targets of current dopamine agonists in PD have been the D₂ and D₃ receptors. Although some findings suggest a greater role for D₁ receptors (Taylor et al., 1991; Mailman et al., 2001), the clinical data of relevance to this topic deals with D₂R-targeted therapeutics.

Investigations into the relationship between D₂R antagonists and cancer began almost as soon as these drugs were approved for psychiatric indications (Table 1), starting with isolated case reports of increased treatment response from cancer patients treated concurrently with antipsychotics (Osterman, 1961; Csatary, 1972; Eicke, 1973; Hercborgs, 1988). Correlative studies of cancer risk in the context of other diseases strengthened this anecdotal association (Fig. 2), (Fig. 3), Table 2). By the 1980s, population-based correlative studies to determine cancer risk within groups of patients with schizophrenia and PD were underway. Many studies showed clear, significant differences in cancer development, yet methodologies were quite variable, and cohorts often small. Some studies were prospective and followed matched cohorts,

Dopamine receptors				
D1-like - Gas coupled		D2-like - Gai/o coupled		
D1	D5	D2	D3	D4
Substantia nigra Nucleus accumbens Olfactory bulb Lower levels: Cerebellum Hippocampus Thalamus Kidney	Substantia nigra Hypothalamus Kidney Heart Sympathetic ganglia	Substantia nigra Nucleus accumbens Ventral tegmental area Lower levels: Heart Blood vessels Adrenal glands Sympathetic ganglia	Olfactory bulb Nucleus accumbens	Heart Blood vessels Substantia nigra Hippocampus Amygdala Gastrointestinal tract

Fig. 1. Dopamine receptors are G protein-coupled receptors, which are divided into the D₁- and D₂-like families. Some tissues of interest where these receptors are expressed are included here.

TABLE 1
Timeline of D₂ receptor pharmacology and early cancer findings

Year	Event	Source
1950	Chlorpromazine synthesized	
1952	Chlorpromazine identified as antipsychotic	Delay et al. (1952), Sigwald and Bouttier (1953)
1959	Reactive oxygen species are associated with antipsychotics at millimolar concentrations.	Dawkins et al. (1959)
1961–1988	First published case reports of increased sensitivity to chemotherapy with concurrent antipsychotic treatment.	Osterman (1961)
1976–1979	Dopamine receptor families proposed (D ₁ -like, D ₂ -like)	Garau et al. (1978), Kebabian and Calne (1979)
1986	Phenothiazines can reverse doxorubicin resistance in KB cells.	Akiyama et al. (1986)
1988–1989	D ₂ -like receptors cloned (human and rodent).	Bunzow et al. (1988)
1990	Pimozide and thioridazine reduce breast cancer cell proliferation	Strobl et al. (1990)
1991	Radiation sensitization of bone marrow under concurrent chlorpromazine treatment.	Jagetia and Ganapathi (1991)
1992	Bromocriptine (D ₂ agonist) increases cancer growth and proliferation (rat gastric carcinogenesis model).	Iishi et al. (1992)
1993	<i>DRD2</i> gene linked to breast cancer via linkage study in a single family lacking <i>BRCA1</i> deficiency.	Cortessis et al. (1993)
1994	D ₂ R are present and inducible by retinoic acid in SH-SY-5Y neuroblastoma cells.	Farooqui et al. (1994)

whereas others mined national healthcare databases. These differences complicate arriving at a unitary hypothesis.

Of particular note was a study of more than 100,000 age- and gender-matched, primarily Han Chinese schizophrenia patients in which both male and female subjects showed a strong inverse correlation for age and development of cancers (Wu et al., 2013). One possible explanation for this trend is that older patient populations had undergone long-term treatment with neuroleptic agents that might have attenuated the increased risk inherent in schizophrenics. This study was limited, however, by the lack of ethnic diversity, as well as the lack of stratification for other risk factors, such as smoking status.

The D₂ Receptor Is Expressed in a Number of Cancer Cell Lines and in Patient Samples. D₂ receptor expression has been reported at both the mRNA and protein levels in a variety of cancers. Increased immunohistochemical staining has been reported in cervical, esophageal, and lung cancers, often correlating with tumor grade or survival (Li et al., 2006; Hoepfner et al., 2015; Kanakis et al., 2015; Mao et al., 2015; Cherubini et al., 2016). In acute myeloid leukemia (AML), D₂R protein is also highly expressed. *DRD2* mRNA levels are elevated in breast cancer (Pornour et al., 2014), ovarian cancer (Moreno-Smith et al., 2011), glioma (Li et al., 2014), and neuroblastoma (Deslauriers et al., 2011). Peripheral blood mononuclear cells of breast cancer patients express *DRD1*, *DRD2*, *DRD3*, and *DRD4* mRNA (Pornour et al., 2014). Because the D₃R and D₄R have significant homology to the D₂R and often recognize the same drugs, these D₂-like receptors may also be relevant.

It is a reasonable hypothesis that tumors derived from cells in which dopamine plays a clear D₂-mediated inhibitory role (e.g., from the pituitary, etc.) would be inhibited by D₂ agonists. Indeed, the early suggestions to this effect (Csatary, 1972; Jacobs and Franks, 1975) have led to the use of dopamine agonists as one mechanism for controlling such tumors, in which a clear role of dopamine receptors can be demonstrated (Hoepfner et al., 2015), sometimes involving effects on angiogenesis (Chauvet et al., 2017). In clinical, and many of laboratory in vivo, studies of such uses of dopamine agonists, the doses used, after allometric adjustment,

are consistent with mediation via the D₂ receptor rather than off-target effects. Yet whereas targeting certain types of tumors with dopamine agonists has a sound physiologic rationale, many

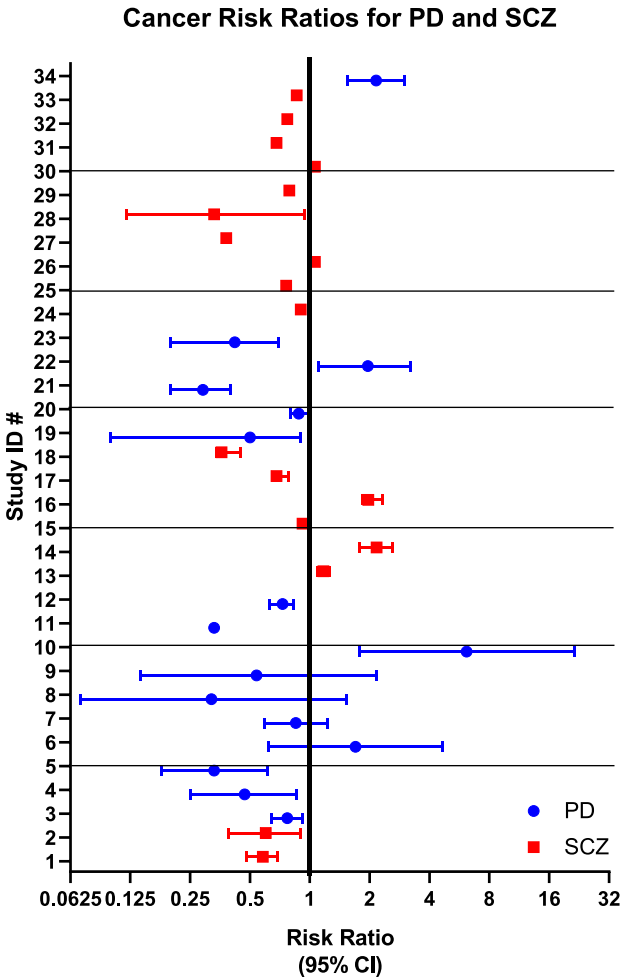


Fig. 2. Forest plot of risk ratios from Table 2, by ID number. Bars represent 95% confidence intervals. Studies of PD patients are shown in blue, and studies of SCZ patients are in red.

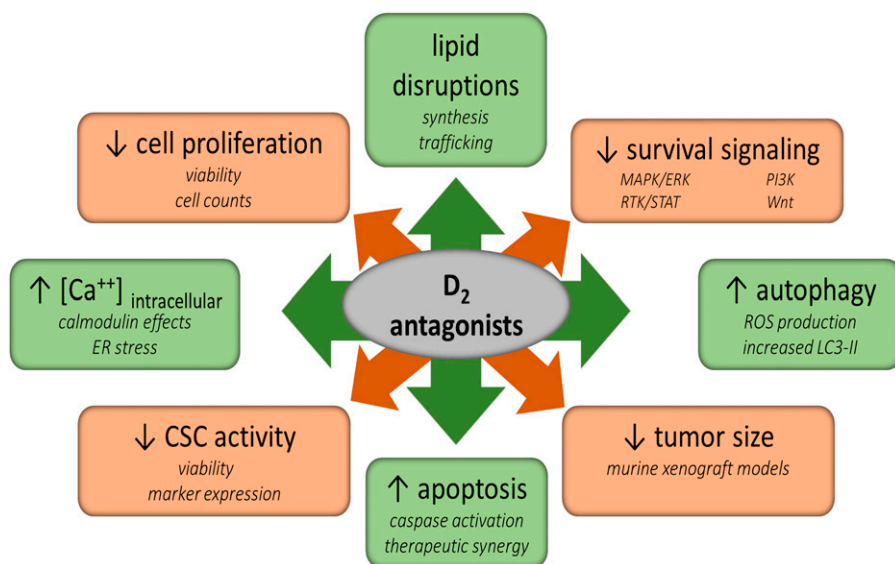


Fig. 3. Treatment with D₂ antagonists affects many vital metabolic processes within cancer cells and tumors. Cancer stem cell-like activities, survival signaling, and proliferation are reduced by treatment. However, intracellular calcium levels, autophagy, and apoptosis are increased. Additionally, lipid synthesis and trafficking are disrupted. The direct mechanisms by which these alterations occur is not currently known, but these compounds may ultimately lead to cell death through these or other pathways.

of the studies ascribing roles for dopamine receptors have important limitations: the use of small numbers of patient samples, lack of blinding, and use of antibodies with poor specificity (Stojanovic et al., 2017). Few studies have ascertained both protein and mRNA levels of the D₂R, and no histochemical studies have published replicate data with other probes to verify selectivity. Importantly, reported mRNA levels have typically been quite low, so large fold-changes in mRNA presence may have little functional impact. Thus, although many studies have reported potential anticancer efficacy of dopamine receptor ligands, a large number have failed to show definitive presence of D₂R protein or message, especially when the drugs being studied were antagonists. We shall explore these important issues below.

Cancer and the “Non-Neuropharmacology” of Dopamine Receptor Ligands

Some of the earliest indications of anticancer activity for D₂R ligands were from Driscoll et al. (1978) and Akiyama et al. (1986). Micromolar concentrations of phenothiazine antipsychotics reversed KB-cell resistance to doxorubicin, vinblastine, dactinomycin, and daunorubicin in a noncalmodulin-dependent manner (Akiyama et al., 1986). In contrast, another study concluded that a reduced proliferative effect of the D₂-like antagonists thioridazine and pimozide in Michigan Cancer Foundation (MCF)-7 cells was attributable to calmodulin antagonism (Strobl et al., 1990). Yet, Iishi et al. (1992) soon reported that the D₂-like agonist bromocriptine promoted gastric carcinogenesis in a rat model, shortly followed by the suggestion of genetic linkage between the *DRD2* gene and BRCA1-sufficient breast cancer (Cortessis et al., 1993). Although these early studies hinted at a potential role for D₂R antagonism in cancer development and treatment, there are some issues that should be considered in interpreting these data. In particular, the effects of the four antipsychotic drugs noted above required concentrations two or more orders-of-magnitude higher than their K_D (Table 3).

Large-Scale Screens Have Identified D₂R as a Potential Target for Anticancer Therapies. Since 2003, several

screening studies identified D₂R antagonists as potential therapeutics for cancer treatment on the basis of their biologic activity and/or presence in cancer cells. Like calmodulin inhibitors, phenothiazines selectively increased Forkhead box (FOXO) transcription factor nuclear localization in 786-O renal cell adenocarcinoma cells (Kau et al., 2003), yet FOXO localization remained unchanged when treated with D₂R antagonists of different chemotypes (i.e., clozapine and haloperidol) to control for off-target effects. Although this suggests that the D₂R is not involved, it contrasts with previous reports noting that D₂R agonist treatment increases phospho-Akt levels in neurons, an effect that would be expected to exclude FOXO from the nucleus (Brami-Cherrier et al., 2002; Kihara et al., 2002). Nuclear localization and transcriptional activity of FOXO3 in the human breast cancer BT549 cell line, however, was increased by 5 μ M-concentrations of the calcium channel blocker bepridil or the antipsychotic trifluoperazine (Park et al., 2016).

An *in silico* screening approach suggested thioridazine may inhibit the Akt/phosphoinositide 3-kinase (PI3K) pathway as well (Rho et al., 2011). Experimentally, thioridazine (20 μ M) decreased PI3K pathway activation, inhibited cell cycle progression at G1, reduced cell viability, and induced apoptosis via caspase-3 cleavage over 24 hours of treatment in a manner that was additive with paclitaxel and cisplatin. This suggested that phenothiazines could impact Akt/PI3K signaling in a cell type-specific manner, but target engagement was not verified and may not involve the D₂R (Rho et al., 2011). More recently, Gutierrez et al. (2014) did dual screening seeking compounds that were toxic toward zebrafish thymocytes that overexpress MYC and synergized with Notch inhibitors in human T-cell acute lymphoblastic leukemia (T-ALL) cells. They identified several phenothiazines (including perphenazine and chlorpromazine) as potential anti-T-ALL treatments that bound protein phosphatase 2A (PP2A) (Gutierrez et al., 2014).

Two large-scale screens identified the D₂R protein itself as a potential target that is upregulated in pancreatic cancer and glioblastoma multiforme. The D₂R and its associated G protein G_{α12} were highly upregulated in pancreatic ductal adenocarcinoma tissue samples (Jandaghi et al., 2016). In an short-hairpin (sh)RNA screen to identify genes necessary for glioblastoma

TABLE 2
Cancer risk in schizophrenia and Parkinson's disease patients

#	Study	Odds Ratio	95% CI	Type	Cancer Type	Cohort (n)	Age	Sex	Diagnosis	Note
1	Barak et al. (2005)	0.58	0.48–0.69	SIR	All types	3226		M/F	SCZ	
2	Barak et al. (2005)	0.6	0.39–0.90	SIR	Breast	1247		F	SCZ	
3	Becker et al. (2010)	0.77	0.64–0.92	IRR	All types	2993		M/F	PD	
4	Becker et al. (2010)	0.47	0.25–0.86	IRR	Lung	2993		M/F	PD	
5	Becker et al. (2010)	0.33	0.18–0.61	IRR	Lymphoma/leukemia	2993		M/F	PD	
6	Becker et al. (2010)	1.7	0.62–4.67	IRR	Melanoma	2993		M/F	PD	
7	Driver et al. (2007)	0.85	0.59–1.22	Adjusted RR	All types	487		M/F	PD	Prospective
8	Driver et al. (2007)	0.32	0.07–1.53	Adjusted RR	Lung	487		M/F	PD	Prospective
9	Driver et al. (2007)	0.54	0.14–2.16	Adjusted RR	Colorectal	487		M/F	PD	Prospective
10	Driver et al. (2007)	6.15	1.77–21.37	Adjusted RR	Melanoma	487		M/F	PD	Prospective
11	Jansson and Jankovic (1985)	~0.33		Combined IR	All types	406		M/F	PD	
12	Jespersen et al. (2016)	0.73	0.63–0.83	Adjusted OR	Prostate	45,429		M	PD	Case control
13	Lichtermann et al. (2001)	1.17	1.09–1.25	SIR	All types	26,996		M/F	SCZ	
14		2.17	1.78–2.6	SIR	Lung	26,996		M/F	SCZ	
15	Wu et al. (2013)	0.92	0.9–0.96	SIR	All types	102,202	All	M/F	SCZ	Not controlled for smoking
16	Wu et al. (2013)	1.97	1.85–2.33	SIR	All types	102,202	20–29	M/F	SCZ	Declines with age
17	Wu et al. (2013)	0.68	0.65–0.78	SIR	All types	102,202	60–69	M/F	SCZ	
18	Wu et al. (2013)	0.36	0.34–0.45	SIR	All types	102,202	>70	M/F	SCZ	
19	Hamaue et al. (2000)	<1		SIR	All types	246		M/F	PD	Retrospective, not significant (small sample size)
20	Moller et al. (1995)	0.88	0.8–1.0	Relative risk	All types	7046		M/F	PD	National cohort
21	Moller et al. (1995)	0.29	0.2–0.4	Relative risk	Lung	7046		M/F	PD	
22	Moller et al. (1995)	1.96	1.1–3.2	Relative risk	Melanoma	7046		M/F	PD	
23	Moller et al. (1995)	0.42	0.2–0.7	Relative risk	Bladder	7046		M/F	PD	
24	Munk-Jorgensen and Mortensen (1989)	0.9		IRR	All types	6152	All	M/F	SCZ	
25	Munk-Jorgensen and Mortensen (1989)	0.76		IRR	All types	2956	All	M	SCZ	
26	Munk-Jorgensen and Mortensen (1989)	1.06		IRR	All types	3196	All	F	SCZ	
27	Munk-Jorgensen and Mortensen (1989)	0.38		IRR	Respiratory	6152	All	M/F	SCZ	
28	Eaton et al. (1992)	0.33	0.12–0.94	IRR	Prostate	38	All	M	SCZ	Treated with high dose neuroleptics (e.g., chlorpromazine)
29	Mortensen (1994)	0.79		SIR	All types	9156	All	M/F	SCZ	With SCZ diagnosis
30	Mortensen (1994)	1.06		SIR	All types	5658		M	SCZ	
31	Mortensen (1994)	0.68		SIR	All types	5658		M	SCZ	Before SCZ diagnosis
32	Mortensen (1994)	0.77		SIR	All types	3498		F	SCZ	Before SCZ diagnosis
33	Mortensen (1994)	0.86		SIR	All types	3498		F	SCZ	
34	Wang et al. (2015)	2.16	1.55–2.99	Adjusted OR	All types	6211	All	M/F	PD	Patients prescribed ergot-derived dopamine agonists

SIR, Standardized Risk Ratio; IRR, Incident Rate Ratio; RR, Relative Risk; OR, Odds Ratio.

TABLE 3
Ligand affinities of select D₂ antagonists, agonists, and functionally selective ligands (nM)¹

Compound	D2	Reference Ligand	Source	D1	Reference Ligand	Source	D3	Reference Ligand	Source	D4	Reference Ligand	Source	D5	Reference Ligand	Source
Aripiprazole	0.59	³ H-NMSP	Lawler et al. (1999)	410	³ H-SCH23390	Lawler et al. (1999)	9.7	³ H-NMSP	Shapiro et al. (2003)	514	³ H-NMSP	Besnard et al. (2012)	1200	³ H-SCH23390	Lawler et al. (1999)
Chlorpromazine	2	³ H-NMSP	Besnard et al. (2012)	112	³ H-SCH23390	Besnard et al. (2012)	1.3	³ H-raclopride	Seeman (1995)	24	³ H-NMSP	Besnard et al. (2012)	133	³ H-SCH23390	Besnard et al. (2012)
Clomipramine	77.6	³ H-spiperone	Millan et al. (2001)	219	³ H-SCH23390	Toll et al. (1998)	50.1	³ H-spiperone	Millan et al. (2001)						
Clozapine	431	³ H-NMSP	Besnard et al. (2012)	189	³ H-SCH23390	Besnard et al. (2012)	340	³ H-nemonapride	Seeman (1995)	39	³ H-NMSP	Besnard et al. (2012)	235	³ H-SCH23390	Besnard et al. (2012)
Fluphenazine	0.54	³ H-NMSP	Besnard et al. (2012)	24	³ H-SCH23390	Besnard et al. (2012)	0.3	³ H-nemonapride	Seeman (1995)	36	³ H-NMSP	Besnard et al. (2012)	12	³ H-SCH23390	Besnard et al. (2012)
Haloperidol	2	³ H-NMSP	Besnard et al. (2012)	83	³ H-SCH23390	Besnard et al. (2012)	23	³ H-nemonapride	Seeman (1995)	15	³ H-NMSP	Besnard et al. (2012)	147	³ H-SCH23390	Besnard et al. (2012)
Olanzapine	72	³ H-NMSP	Besnard et al. (2012)	58	³ H-SCH23390	Besnard et al. (2012)	40	³ H-nemonipride	Seeman (1995)	19	³ H-NMSP	Besnard et al. (2012)	90	³ H-SCH23390	Besnard et al. (2012)
Penfluridol	5.6	³ H-haloperidol	Burt et al. (1976)	1600*	³ H-dopamine	Burt et al. (1976)				31**	³ H-spiperone	Roth et al. (1995)			
Perphenazine	1.4	³ H-NMSP	Burt et al. (1976), Kroeze et al. (2003)	29.9**	³ H-SCH23390	Billard et al. (1984)	1.1	³ H-nemonapride	Seeman (1995)	32	³ H-spiperone	Seeman (1995)			
Pimozide	0.65	³ H-NMSP	Kroeze et al. (2003)	>10 ⁴	³ H-SCH23390	Toll et al. (1998)	11	¹²⁵ I-iodosulpride	Schmidt et al. (1992)	1.8	R-SAT	Burstein et al. (2005)			
Prochlorperazine	4	³ H-spiperone	Seeman (1995)				1.8	¹²⁵ I-iodosulpride	Schmidt et al. (1992)	70	³ H-spiperone	Seeman (1995)			
Risperidone	4.9	³ H-NMSP	Besnard et al. (2012)	60.6	³ H-SCH23390	Besnard et al. (2012)	5.2	³ H-nemonapride	Seeman (1995)	18.6	³ H-NMSP	Besnard et al. (2012)	16	³ H-SCH23390	Besnard et al. (2012)
SCH 23390	2145	³ H-domperidone	Grandy et al. (1989)	0.35	³ H-SCH23390	Sumahara et al. (1991)	>10,000	³ H-nemonapride	Neumeyer et al. (2003)	3560	³ H-spiperone	Van Tol et al. (1991)	0.3	³ H-SCH23390	Sumahara et al. (1991)
Spiperone	0.125	³ H-domperidone	Grandy et al. (1989)	577	³ H-SCH23390	Sumahara et al. (1991), Toll et al. (1998)	0.275	³ H-spiperone	Cussac et al. (2000), Neumeyer et al. (2003)	4	³ H-spiperone	Tang et al. (1994)	4500	³ H-SCH23390	Sumahara et al. (1991)
Thioridazine	10	³ H-NMSP	Grandy et al. (1989), Besnard et al. (2012)	89	³ H-SCH23390	Besnard et al. (2012)	5.2	³ H-spiperone	Seeman (1995)	17	³ H-NMSP	Besnard et al. (2012)	216	³ H-SCH23390	Besnard et al. (2012)
Trifluoperazine	1.3	³ H-NMSP	Kroeze et al. (2003)	740*	³ H-dopamine	Burt et al. (1976)				44	³ H-spiperone	Seeman et al. (1997), Besnard et al. (2012)			

Unmarked, human receptor. *, calf receptor. **, rat receptor.

¹Most values and references from the PDSP K_i database (<https://pdsp.uncc.edu/databases/pdsp.php>) except for Seeman (1995) and Lawler et al. (1999).

(GBM) cell line survival, the D₂R was also identified (Li et al., 2014). Inhibition of D₂R signaling with shRNA, short-interfering (si)RNA, and several antagonists (i.e., spiperone, haloperidol, risperidone, and L-741,626) reduced cell viability, proliferation, and clonogenicity in U87MG glioblastoma cells. To our knowledge, this was the only study to show that *DRD2* knockdown reduces cell viability and tumor growth.

D₂R Antagonists Reduce Cell Proliferation and Induce Apoptosis In Vitro. During the past 20 years, other studies also have identified D₂R antagonists as potential anticancer therapeutics through in vitro studies utilizing cell lines and patient samples (Table 4). Phenothiazines, most notably thioridazine, have been suggested as anticancer therapeutics more often than other chemotypes, but haloperidol, pimozide, and olanzapine also have been studied. These compounds have been shown to reduce cell viability, induce apoptosis, cause necrotic cell death, induce cell-cycle arrest, and alter protease activity (Fig. 1). This anticancer activity is apparent in a broad range of cancer types, including gender-specific (Kang et al., 2012; Mao et al., 2015; Park et al., 2016; Ranjan and Srivastava, 2016; Ranjan et al., 2016; Zhou et al., 2016), pancreatic (Ranjan and Srivastava, 2016), nervous system (Gil-Ad et al., 2004; Daley et al., 2005; Levkovitz et al., 2005; Shin et al., 2012, 2013; Li et al., 2014; Karpel-Massler et al., 2015), blood (Zhelev et al., 2004), oral (Choi et al., 2014), lung (Yue et al., 2016), gastric (Mu et al., 2014), and renal (Min et al., 2014) cancers, among others (Levkovitz et al., 2005; Nagel et al., 2012). Typical in vitro cell viability assay IC₅₀ values for D₂R antagonists range from 5 to 20 μ M, yet D₂R antagonists appear to be only modestly selective for cancer cells. Fibroblasts were less sensitive to pimozide treatment than five different pancreatic cancer cell lines, but there was a trivial difference in IC₅₀ (2-fold selectivity, 10 vs. 20 μ M) (Jandaghi et al., 2016). Astrocytic cell lines were also less sensitive to haloperidol compared with GBM cells (Li et al., 2014). These concentrations exceed the known maximum tolerated plasma concentrations in humans (Table 5) and suggest a narrow therapeutic window or even dose-limiting toxicity if applied to clinical use. In most cases, cytotoxic concentrations of these compounds are much higher (>100-fold) than would be expected for a D₂R-based mechanism, as determined from D₂R receptor affinity (Table 3). It is possible that this is owing to differences in receptor environment or functional partners, but it is also important to consider other mechanisms, especially because of the multiple targets that high concentrations of these drugs might engage (Besnard et al., 2012).

In Vivo Models of Cancer Suggest Efficacy of D₂R Antagonism. Animal models of cancer have suggested that D₂R antagonists might have chemotherapeutic utility (Table 6). Authors have reported significant reductions in tumor growth with D₂R antagonist treatment in gastric, glial, ovarian, medulloblastoma, oral, lung, pancreatic, prostate, and breast cancer xenograft models. Many of these studies observed evidence of Akt-signaling inhibition and/or alterations in autophagic flux in vivo. In an OVCAR-3 murine xenograft model, 10 mg/kg of thioridazine, trifluoperazine, or chlorpromazine reduced tumor growth, but an equivalent dose of fluphenazine was found to be toxic to the animals (Choi et al., 2008), again suggesting a narrow therapeutic window. A dose of 300 μ g/day thioridazine or 400 μ g/day mepazine reduced tumor size by half in OCI-Ly10 but not in Su-DHL-6 xenograft models (Nagel et al., 2012). These doses led to

compound plasma levels of 200 ng/ml, well below the achievable plasma level of 2000 ng/ml in humans.

In summary, many animal studies have suggested that D₂R antagonists are efficacious in reducing tumor size and prolonging survival in xenograft models. In general, plasma and tumor drug concentrations were not quantified, but they may be expected to be well above selective concentrations. When measured in one study, plasma levels were, however, less than those achievable in human patients (Table 5) (Nagel et al., 2012). Unfortunately, toxicity of some compounds was observed. Therefore, D₂ receptor involvement is difficult to ascertain solely on the basis of pharmacological data. Ideally, such findings would be corroborated by studies that employed genetic methods to identify a target. To our knowledge, only one study probed the role of *DRD2* in a xenograft model in this way. In this study, a doxycycline-inducible *DRD2* knockout in U87MG intracranial xenografts prevented tumor growth in Nu/Nu mice, providing strong support for a role of D₂R in cancer growth (Li et al., 2014). Although most of these studies were carried out in the context of immunodeficient mice, it is tempting to speculate on the effects that D₂R modulators may have on the immune system through both indirect and direct means (i.e., through psychoactive effects or through direct interaction with immune cells).

D₂R Antagonists Are Associated with Anti-Cancer Stem Cell Activity. D₂R expression is also implicated in stem-like cells [cancer stem cells (CSCs)], hypothesized slow-cycling cells that promote tumor growth, chemoresistance, and metastasis. One in silico study using the Connectivity Map identified phenothiazines, notably trifluoperazine, as potential therapeutic agents capable of reversing stem-like gene expression profiles (Yeh et al., 2012). Trifluoperazine concentration-dependently induced apoptosis in a patient-derived, gefitinib-resistant lung cancer cell line, and reduced clonogenicity in a number of other patient-derived lines, regardless of epidermal growth factor receptor (EGFR) status. In a green fluorescent protein (GFP) reporter-based screen for Oct4 and Sox2 in human neoplastic pluripotent stem cells (hnPSCs), thioridazine appeared to target CSCs with an EC₅₀ of 7 μ M; prochlorperazine and fluphenazine were also identified, but not further characterized in this work (Sachlos et al., 2012). D₂R antagonists, including thioridazine (10 μ M), reduced cell number and colony-forming units in AML samples and hPSCs (Sachlos et al., 2012). This work was the first to conclude that D₂R activity contributes to the survival and function of CSCs and employed both agonists and antagonists to examine this possibility. In glioblastoma CSCs, similar results were seen for the D₂R functionally selective, partial agonist aripiprazole (10 μ M) (Suzuki et al., 2016) as well as the D₂R antagonists thioridazine and trifluoperazine and the selective D₄ antagonists PNU 96415E and L-741,742 (Dolma et al., 2016). Taken together, all of these results suggest that D₂R is expressed in CSCs and may impact stemness.

D₂R Receptor Signaling Mechanisms and Cancer Cell Growth

STAT and RTK Signaling. Signal transducer and activator of transcription (STAT) proteins are attractive therapeutic targets because of their role in cellular proliferation and angiogenesis. In a screen for potential STAT5 inhibitors using chronic myelogenous leukemia cell lines, the D₂R antagonist

TABLE 4
D₂ ligand IC₅₀ values in cell culture

Compound	IC ₅₀ (μM)	Experiment Type	Cell Line	Source	Note
Aripiprazole	10–100	Trypan blue	PANC-1, PSN-1, A549	Ikai et al. (2016)	Found autophagy induction
Chlorpromazine	5–10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Chlorpromazine	4.8–14.5	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Chlorpromazine	8	CellTiter-Blue	KOPT-K1	Gutierrez et al. (2014)	
Chlorpromazine	10	CKK-8	U87 MG	Shin et al. (2013)	
Chlorpromazine	~10	CellTiter-Glo	K-562, Daudi, Raji, BALL-1, MOLT-4, HPB-ALL, CCRF-HSB-2	Zhelev et al. (2004)	Cancer stem cell selective (8-fold selectivity)
Fluphenazine	5–10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Fluphenazine	3.9–7.9	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Haloperidol	>25	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Haloperidol	5–15	Clonogenicity	LN18, U87MG, T98G	Li et al. (2014)	
L-741,742	≥50	MTT	BJ, U2OS, Daoy	Dolma et al. (2016)	Cancer stem cell selective (8-fold selectivity)
L-741,742	1.56	MTT	G380	Dolma et al. (2016)	
Olanzapine	25–79.9	MTT	U87MG, A172, SC38, SC40	Karpel-Massler et al. (2015)	
Penfluridol	~5	MTT	MDA-MB-231, HCC-1806, 4T1	Ranjan and Srivastava (2016)	
Penfluridol	3, 4, 5	MTT	Panc-1, AsPC-1, BxPC-3	Ranjan and Srivastava (2016)	
Perphenazine	5–10	MTT	U87MG, GBM8401	Cheng et al. (2015)	Cancer stem cell selective (eightfold selectivity)
Perphenazine	7	CellTiter-Blue	KOPT-K1	Gutierrez et al. (2014)	
Pimozide	7–15	Sulforhodamine B	Panc-1, CFPAC-1, Capan-1, BxPC-3, MiaPaCa-2	Jandaghi et al. (2016)	
Pimozide	~10	MTT	LNcap, PC3M, 22RV1	Zhou et al. (2016)	
Pnu 96415e	>50	MTT	BJ, U2OS, Daoy	Dolma et al. (2016)	
Pnu 96415e	1.56	MTT	G380	Dolma et al. (2016)	Cancer stem cell selective (eightfold selectivity)
Prochlorperazine	>10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Thioridazine	5–10	Crystal violet	NCL-N87, AGS	Mu et al. (2014)	
Thioridazine	~10	CellTiter-Glo	K-562, Daudi, Raji, BALL-1, MOLT-4, HPB-ALL, CCRF-HSB-2	Zhelev et al. (2004)	
Thioridazine	5–10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Thioridazine	12.5–17.5	EZ-Cy Tox	KB, KBV20C	Choi et al. (2014)	Slowed growth over 96 h at 10 μm
Thioridazine	3.8–6.7	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Thioridazine	11.2–15.1	Neutral red/ alamarBlue	C6, SHSY-5Y	Gil-Ad et al. (2004)	
Thioridazine	41.3	Neutral red/ alamarBlue	primary mouse brain	Gil-Ad et al. (2004)	
Thioridazine	~15	MTT	HeLa, C33A, Caski, HEC-1-A, KLE	Byun et al. (2012)	Slowed growth over 96 h at 10 μm
Thioridazine		MTT	SiHa	Mao et al. (2015)	
Thioridazine		MTT	ISK, KLE	Meng et al. (2016)	
Thioridazine				Min et al. (2014)	
Thioridazine				Min et al. (2014)	
Thioridazine	~15	MTT	NCL-N87, AGS	Mu et al. (2014)	Sensitized to TRAIL at 10 μm
Thioridazine	~10	MTT	ABC-DLBCL lines (HBL-1, Ocl-Ly3, U2932, TMD8)	Nagel et al. (2012)	
Thioridazine	>10	MTT	GCB-DLBCL lines (BJAB, Su-DHL-6, Su-DHL-4)	Nagel et al. (2012)	
Thioridazine	20	MTT	SKOV-3	Rho et al. (2011)	
Thioridazine	1–10	CFU	AML blasts	Sachlos et al. (2012)	
Thioridazine	10–20	Flow cytometry (Annexin V/PI)	4T1	Yin et al. (2015)	Also reduces stemness
Thioridazine	~10	MTT	NCL-H1299, 95-D	Yue et al. (2016)	
Trifluoperazine	>10	MTT	U87MG, GBM8401	Cheng et al. (2015)	
Trifluoperazine	4.3–7.7	MTT	SK-MEL-28, HT29, Colo205, SW480, HCT116, MCF7	Choi et al. (2008)	
Trifluoperazine	~7	MTT	MDA-MB-231, BT549	Park et al. (2016)	
Trifluoperazine	10–15	MTT	Ca922	Wu et al. (2016)	Also reduces stemness
Trifluoperazine	>10	MTT	A549, H1975	Yeh et al. (2012)	
Trifluoperazine	~10	CellTiter-Glo	K-562, Daudi, Raji, BALL-1, MOLT-4, HPB-ALL, CCRF-HSB-2	Zhelev et al. (2004)	
Trifluoperazine	5–10	MTT	H69, U1285, U-1906, U-2020	Zong et al. (2014)	

TABLE 5

Tolerated human plasma levels of selected D₂ antagonists

Compound	[C] plasma in humans (nM)		Source
	Max	Min	
Thioridazine	2699	270	Smith et al. (1984)
Chlorpromazine	1548	101	Chetty et al. (1999)
Pimozide	32	2	Kerbusch et al. (1997)
Olanzapine	40	31	Kassahun et al. (1997)
Haloperidol	67	11	Froemming et al. (1989)
Clozapine	4525	1007	Guitton et al. (1998)

pimozide (5–10 μ M) decreased STAT5 phosphorylation and function, even downstream of potent oncogenic activation (Nelson et al., 2011). Moreover, pimozide inhibited IL-6-induced growth and migration via inhibition of STAT3 in prostate cancer cells (Zhou et al., 2016). It is unknown if other D₂R antagonists inhibit STAT directly, but they reduce proneoplastic receptor tyrosine kinase (RTK) signaling upstream of Janus kinase (JAK)/STAT. The D₂R agonists quinpirole (10 μ M) and pramipexole (10 μ M) both increased phosphorylation of extracellular signal-regulated kinases (ERK) and RTK EGFR in a D₂R-dependent manner (Yoon and Baik, 2013). Antagonism with thioridazine reduced vascular EGFR (VEGFR) phosphorylation and VEGF availability (Park et al., 2014). These studies suggest an RTK/JAK/STAT mechanism or downstream effect of D₂R antagonists and a possible role for D₂R in proneoplastic EGFR signaling.

Wnt. The wingless/integrated (Wnt) pathway affects development, carcinogenesis, and stem-like behavior, and is reportedly inhibited by D₂R antagonists. In a patient-derived lung cancer cell line, trifluoperazine concentration-dependently inhibited T-cell factor–mediated transcription (Yeh et al., 2012), with the decreases in Wnt signaling being concomitant with the induction of cytotoxicity. Such findings are supported by an *in silico* docking and network analysis study identifying the Wnt pathway protein glycogen synthase kinase (GSK3 β) as potentially affected by phenothiazine treatment (Qi and Ding, 2013). Spiperone (10 μ M) had similar effects, but these were not mediated by D₂R, serotonin, or $\sigma_{1/2}$ receptor activity by comparison with selective receptor ligands but may have involved intracellular calcium signaling and protein kinase C (PKC) (Lu and Carson, 2009). Furthermore, D₂R and Wnt5a coimmunoprecipitated from HEK293T cells with a K_i of 165 nM for competition with [³H]-spiperone, suggesting a possible direct interaction (Yoon et al., 2011). The quinpirole-induced upregulation of Wnt pathway protein Dvl-3 induces ERK activation in mesencephalic neuronal culture but did not occur using cells from D₂R^{-/-} mice (Yoon et al., 2011). These data suggest that the D₂R may interact with the Wnt pathway in neuronal cells and that D₂R antagonists can decrease Wnt signaling, but further studies are needed to see if this is more broadly applicable to the malignant phenotype.

PI3K. The PI3K/Akt pathway, a critical regulator of the cell cycle, has been suggested as a target pathway for D₂R antagonists in cancer-related cell lines. In Chinese hamster ovary (CHO) cells expressing the human D₂R, dopamine and quinelorane activated the PI3K pathway by increasing phospho-Akt (at both Ser-473 and Thr-308) and GSK-3 β (at Ser-9) levels, with maximal effects at 10 μ M (Mannoury la Cour et al., 2011). Pertussis toxin, as well as D₂R antagonists, blocked this, suggesting a dependence on D₂R G protein signaling. When

receptor internalization was blocked with phenylarsine oxide, phosphorylation levels were reduced by half. Likewise, disruption of cholesterol-rich lipid rafts with methyl- β -cyclodextrin inhibited phosphorylation. These latter data suggest that both G-protein and β -arrestin signaling are important. Increased Akt phosphorylation was PKC- and calmodulin-dependent, and GSK-3 β phosphorylation was attributable, at least in part, to Akt activity. Thus, there is the potential for these mechanisms to affect cancer cell growth, proliferation, and metabolism via Akt downstream effectors, including transcription factors (like FOXO). *In vivo*, 25 mg/kg thioridazine given every third day to 2774-xenografted (ovarian cancer) nude mice reduced phosphorylation levels of PI3K, Akt, pyruvate dehydrogenase kinase 1 (PDK1), and mammalian target of rapamycin (mTOR) (Park et al., 2014). In normal rat brain, however, D₂R antagonist raclopride (3 mg/kg per day) enhanced phosphorylation at both Thr³⁰⁸ and Ser⁴⁷³ of Akt, which indicates activation, but did not alter total Akt protein levels. In the same model, agonist quinpirole reduced phosphorylation (Sutton and Rushlow, 2012). In normal brain, Akt phosphorylation is reduced by D₂-receptor activation in a β -arrestin-2 (β Arr2)-mediated manner involving a complex with PP2A (Sotnikova et al., 2005). Antagonism may increase the overall level of Akt phosphorylation or block cell sensitivity to β Arr2-mediated Akt regulation (Beaulieu et al., 2004). D₂R^{-/-} mouse striatal lysates have increased Akt phosphorylation at Thr-308 both basally and in response to amphetamine (3 mg/kg) challenge (Beaulieu et al., 2007). Overall, it appears PI3K signaling is increased by D₂R agonists but reduced by D₂R antagonists in malignant tissues, whereas the opposite may be true in normal tissues.

Thioridazine (15 μ M) induced apoptosis and inhibited the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway in endometrial and cervical cancer cell lines (Kang et al., 2012), and at similar concentrations had effects resembling PI3K/Akt inhibition (Rho et al., 2011), decreasing PI3K activity by 60%, inducing G1 arrest after 24-hour treatment, reducing cell viability by half at 48 hours, and inducing apoptosis. Phosphorylation of Akt, mTOR, and GSK-3 β were also reduced by several antidopaminergic phenothiazine drugs at low micromolar concentrations in EGF-stimulated OVCAR-3 ovarian cancer cells, although the concentration-response relationship did not parallel D₂R affinity (Choi et al., 2008). PI3K activation was unaffected by these phenothiazines.

MAPK/ERK. The mitogen-activated protein kinase (MAPK)/ERK pathway, known to be involved in cancer cell survival and proliferation, was inhibited in U87MG and A172 glioma cell lines by four different D₂R antagonists, albeit at relatively high concentrations (spiperone and haloperidol at 5 μ M, risperidone and L-741,626 at 10 μ M) (Li et al., 2014). MAPK8 and MAPK10 were also identified as potential targets by a correlational *in silico* docking and network analysis study of phenothiazines, including chlorpromazine, fluphenazine, and trifluoperazine (Qi and Ding, 2013). This may involve a cascade wherein interaction with peroxisome proliferator-activated receptors γ affects MAPK8 status, leading to a protein kinase-modulated alteration of activity in downstream effectors cyclin-dependent kinase 2 and GSK3 β (see section on Wnt signaling). In normal rat and mouse brain slices, the D₂ agonist quinpirole (60 μ M) increased MAPK and cAMP response element-binding protein phosphorylation,

TABLE 6
D₂ antagonist efficacy in animal studies

Compound	Dose (mg/kg)	Timing	Model	Efficacy	Source	Note
Bromocriptine	1 to 2	QD	MNNG-induced Wistar rats (gastric cancer)	~2.5-Fold increase in tumor number	Iishi et al. (1992)	Agonist
Chlorpromazine	10	QD×5	OV/CAR-3 Xenograft, nude mice	64% Tumor growth suppression	Choi et al. (2008)	Synergy with AG1478
Chlorpromazine	20	QD	U87MG Xenograft	43.5% Inhibition of tumor growth	Shin et al. (2013)	
Fluphenazine	10	QD×5	OV/CAR-3 Xenograft, nude mice	Toxicity - ND	Choi et al. (2008)	
Haloperidol	10	QD	MiaPaCa-2 Xenograft in NSG mice	~50% Decrease in tumor mass	Jandaghi et al. (2016)	
Haloperidol	10	QD	U87MG Xenograft	No significant reductions in tumor size or survival	Li et al. (2014)	
Imipramine	40	QD	GR1p53het Mice	2-Fold increase in survival time over control	Shchors et al. (2015)	Cooperated with ticlopidine to enhance autophagy and increase survival
L-741,742	20	QD	G362 Xenograft, flank and intracranial	40.9% Reduction in tumor mass, prolonged survival	Dolma et al. (2016)	Synergy with TMZ
Penfluridol	10	QD	4T1 Orthotopic mammary xenografts, female Balb/c mice	49% Reduction in tumor size	Ranjan and Srivastava (2016)	
Penfluridol	10	QD	4T1-luc Intracardiac metastasis model, female Balb/c	90% Reduction in brain fluorescence from luciferase reporter	Ranjan and Srivastava (2016)	
Penfluridol	10	QD	4T1 Intracranial xenograft	~33% Reduction in brain fluorescence from luciferase reporter	Ranjan and Srivastava (2016)	
Penfluridol	10	QD	BxPC-3 Xenografts (subcutaneous) in athymic nude mice	~50% Reduction in tumor volume at day 27	Ranjan and Srivastava (2016)	
Perphenazine	10	QD	hTALL2 Cells in NSG mice	~33% Reduction in luciferase bioluminescence	Gutierrez et al. (2014)	Agonist
Pnu 96415e	20	QD	G362 Xenograft, flank	44.3% Reduction in tumor mass	Dolma et al. (2016)	
Quinpirole	10	QD	LLC1 Xenograft	~66% Reduction in bioluminescence	Hoepfner et al. (2015)	
Thioridazine	10	QD×5	OV/CAR-3 Xenograft, nude mice	26% Tumor growth suppression	Choi et al. (2008)	
Thioridazine	Pretreatment of cells		NCL-N87 Xenograft	Slight, insignificant reductions in tumor size or survival	Mu et al. (2014)	
Thioridazine	300 µg/animal	QD	Ocl-Ly10 Xenograft, nude mice		Nagel et al. (2012)	Pretreatment with 5 µm thioridazine
Thioridazine	25	QD	2774 Xenografts, nude mice	>50% Reduction in tumor size and volume, significantly reduced Ki67 staining	Park et al. (2014)	Oral delivery
Thioridazine	32	QD	4T1 Xenograft in BALB/c NCI-H1299	55% Reduction in tumor volume	Yin et al. (2015)	
Thioridazine	Pretreatment of cells			~50% Reduction in tumor size at day 47	Yue et al. (2016)	
Trifluoperazine	10	2×/wk	MDA-MB-231 Xenografts, nude mice	~50% Reduction in tumor volume at day 33	Park et al. (2016)	
Trifluoperazine	5	QD	CL97 Tail vein injection NOD/SCID	Significant reduction in bioluminescence/tumor size	Yeh et al. (2012)	
Trifluoperazine	10	QD×5	OV/CAR-3 Xenograft, nude mice	46% Tumor growth suppression	Choi et al. (2008)	

with effects blocked by the D₂ antagonist eticlopride (40 μ M), the calcium chelator BAPTA-AM, or the PKC antagonist Go6976 (Yan et al., 1999). Although these investigators did not directly assay G protein activity, they hypothesized a role for G α_q activation (Yan et al., 1999), although the D₂-like receptors normally are not considered to couple readily to this α -subunit. Owing to the heterogeneous nature of the system and use of healthy tissue, these findings may or may not have any relationship to the behavior of cancer cells exposed to ligands that modulate D₂R function.

Calcium Signaling. D₂R signaling and antagonist treatments both alter calcium signaling. Wolfe and Morris (1999) found that both the long and short D₂R isoforms interacted with G α_o to reduce high-voltage-activated calcium channel activity. In wild-type astroglia, dopamine signaling is capable of both increasing and reducing intracellular calcium levels in a manner dependent on local neural type in brain slices (Jennings et al., 2017). Dopamine D₂/D₃ receptors were involved in the negative regulation of Ca²⁺ in this study.

The calcium channel blocker bepridil and the D₂R antagonist triflupromazine had similar effects on PI3K signaling through FOXO3 in MDA-MB-231 breast cancer cells (Park et al., 2016). FOXO3 activity was required to reduce colony formation with both trifluoperazine and bepridil, and FOXO3-regulated proteins D₂R, KLF-5, and c-Myc were downregulated by treatment with either drug. In vivo, 10 mg/kg trifluoperazine or bepridil three times a week significantly reduced tumor volume of MDA-MB-231 xenografts in female athymic (nu/nu) mice (Park et al., 2016). A calmodulin mechanism was posited for both compounds but not explored experimentally.

In pancreatic cancer lines MiaPaCa-2 and Panc-1, 10 μ M pimozide, or L-741,626 increased intracellular calcium levels sharply within seconds of treatment and concentration-dependently increased phospho-protein kinase R-like endoplasmic reticulum kinase (PERK), suggesting an increase in endoplasmic reticulum stress (Jandaghi et al., 2016). PKA phosphorylation activity was also modestly increased. Caspase activity upon treatment with pimozide was reduced by around 25% when ATF4 was silenced with shRNA, further supporting the involvement of the unfolded protein response. Similar results were found for haloperidol, except IC₅₀ values were increased and fibroblasts seemed even more resistant. Overall, it appears that multiple chemotypes of D₂R antagonists can alter intracellular calcium levels and initiate cellular stress in cancer cells.

Autophagy May Be Affected by D₂ Antagonists. Numerous studies have suggested that D₂R antagonists are able to induce autophagic cell death in the context of in vitro and in vivo studies of cancer. One trifluoperazine derivative, A4, increased reactive oxygen species (ROS), DNA damage, and autophagic cell death, while also causing apoptosis and activating AMP-activated protein kinase K (AMPK) (Wu et al., 2016). AMPK phosphorylation increases were also seen in D₂R antagonist-treated GBM stem cell cultures (Cheng et al., 2015). In SH-SY-5Y neuroblastoma cells, sertindole, pimozide, and trifluoperazine were identified as autophagy-inducing agents by a large-scale fluorescence-based screen (Shin et al., 2012). Increases in GFP-LC3 puncta were sertindole concentration- and time- dependent; autophagosome formation was also verified by electron microscopy. LC3 cleavage was responsive to GFP 3-methyladenine, suggesting autophagic induction was partially regulated by the PI3K pathway. Conditional

siRNA knockdown of the essential autophagic protein, ATG5, reduced autophagosome formation, enhanced cell viability, and reduced LC3 cleavage under treatment with 10 μ M sertindole. A fluorescence assay that included ROS scavengers indicated a partial role for ROS in the cytotoxicity of sertindole. Similar results have been reported in glioma cell lines (Shin et al., 2013; Cheng et al., 2015). Although autophagy can contribute to D₂ antagonist-mediated cell death, D₂ activity does not appear to be involved in this mechanism since thioridazine reduced D₂R protein levels and increased autophagy, whereas trifluoperazine reduced D₂R protein levels and did not increase autophagy at the same concentrations.

Lipid Synthesis and Trafficking Is Altered by D₂R Antagonist Treatment. An early study reported that chlorpromazine (10 μ M) inhibited both sphingomyelinase activity and esterification of cholesterol in human fibroblasts in a manner comparable to 10 μ M W-7, a known calmodulin antagonist (Masson et al., 1992). Chlorpromazine treatment resulted in accumulation of unesterified cholesterol in lysosomal vacuoles reminiscent of a Niemann-Pick type C (NPC) lipidosis phenotype (Masson et al., 1992). Similar results were seen with 10–50 μ M haloperidol, and concomitant insulin receptor signaling inhibition was reversed by cholesterol add-back, suggesting lipid raft disruption (Sanchez-Wandelmer et al., 2010).

Other antipsychotics like haloperidol (10 μ M) and clozapine (30 μ M) increased cholesterol and fatty acid synthesis enzyme mRNA by 2- to 4-fold in GaMg glioma cells at 5–10 hours (Ferno et al., 2006). Sterol regulatory element-binding proteins (SREBP)-1 and SREBP-2, sterol-responsive transcription factors that regulate these genes, were upregulated at the protein level, supporting the idea that antipsychotic treatment may upregulate lipogenesis via SREBP signaling. Cholesterol-related mRNAs, including 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), apolipoprotein E (APOE), ATP binding cassette subfamily A member 1 (ABCA1), liver X receptors (LXR) α/β , and NPC1/2, were increased after 24- to 48-hour treatment with clozapine (25 μ M), haloperidol (10 μ M), olanzapine (10 μ M), or imipramine in GaMg cells (Vik-Mo et al., 2009). Protein levels of apoE also increased in GaMg and HepG2 human hepatocellular carcinoma cells. Message-level increases were more striking in glial cell cultures, suggesting the activation of LXR and its downstream targets may occur as an effect of earlier SREBP-modulated lipogenesis within the cell (Ferno et al., 2006). Lipogenesis and adequate cholesterol stores are essential for cancer cell survival, particularly in the case of gliomas, which are highly sensitive to exogenous cholesterol levels and LXR activity (Villa et al., 2016).

Although haloperidol and pimozide treatment (10 μ M) slightly increased the expression of some SREBP-responsive genes, they also disrupted cholesterol trafficking, causing intracellular accumulation of unesterified cholesterol in intracellular puncta in CHO-7 cells (Kristiana et al., 2010). Despite increases in active SREBP-2, cholesterol synthesis was ablated under treatment with these compounds. Aripiprazole, clozapine, quetiapine (all 10 μ M), olanzapine, risperidone, and ziprasidone (25 μ M) showed similar behavior, suggesting that the effect may be mediated by D₂R or another common target of these compounds. Kristiana et al. (2010) posited that the intracellular trafficking of cholesterol was disrupted by these drugs, inhibiting SREBP cleavage-activating

protein (SCAP) activation of SREBP and sterol *O*-acyltransferase 1 (SOAT-1) esterification of cholesterol. Likewise, 10–50 μ M haloperidol reduces biosynthesis of cholesterol in SH-SY-5Y cells while generating a buildup of sterol precursors (Sanchez-Wandelmer et al., 2010). Risperidone, ziprasidone, and clozapine (5–25 μ M) also induced buildup of sterol intermediates in HepG2 cells (Canfran-Duque et al., 2013).

Clearly, numerous chemotypes of D₂R antagonists can reduce cellular cholesterol levels, disrupt lipid rafts, and alter lipid trafficking. These effects, however, have not been shown to be the cause of D₂R antagonist-induced cytotoxicity; it is possible that lipid alterations result from cellular coping mechanisms for other types of stress, such as ROS or autophagic stress. Indeed, these lipid phenotypes indicate that cancer cells treated with these compounds behave as though they are lipid-starved and frustrated in their attempts to synthesize more. One final point should be noted—many (but not all) of the D₂-like antagonists used clinically can cause a metabolic syndrome that can include hyperlipidemia (Hirsch et al., 2017; Hoffman, 2017), yet the latter effects require chronic use of the antipsychotics, and the drug concentrations in human tissue are far lower than those causing anticancer effects in vitro or in animals. These factors suggest that different mechanisms probably are involved.

D₂R Antagonists May Interact Positively with Other Compounds to Increase Their Anticancer Efficacy.

Studies also indicate that D₂R antagonists can be additive with common chemotherapeutics. Aripiprazole sensitized CSC-enriched cultures to gemcitabine, 5-FU, and cisplatin treatment in an additive manner (Suzuki et al., 2016). Likewise, the proapoptotic effects of trifluoperazine were synergistic with cisplatin (10 μ M) and gefitinib (2.5–10 μ M) in a patient-derived lung cancer cell line (Yeh et al., 2012). Tumor volume and weight of G362 GBM xenografts were decreased in mice treated with 20 mg/kg of either PNU 96415E or L-741,742 over control, though the difference in size was not large (Dolma et al., 2016). L-741,742 treatment on its own failed to improve survival of xenografted mice, but survival increased under cotreatment with temozolomide over treatment with temozolomide alone. Likewise, thioridazine increased the efficacy of AraC in leukemia (Dolma et al., 2016), and cisplatin or paclitaxel in ovarian cancer (Rho et al., 2011). In treatment-resistant endometrial cancer cell lines ISK and KLE, combination treatment with 20 μ M medroxyprogesterone acetate and 10 μ M thioridazine reduced cell viability by half after 4 days (Meng et al., 2016). Such observations could potentially be explained by inhibition of P-glycoprotein or other efflux pumps associated with drug resistance, as suggested by the fact that thioridazine sensitizes chemoresistant oral squamous cancer cells (KBV20C) to vinblastine owing to inhibited P-glycoprotein efflux (Choi et al., 2014). Likewise, ABCG2-mediated chemoresistance in MDR cells is reduced by 10 μ M D₃ antagonists PG01037, NGB 2904, SB27 7011A, and U99194 (Hussein et al., 2017). Hussein et al. (2018) later reported that cariprazine (which they termed a D₂/D₃ partial agonist) had similar effects and suggested it might be repurposed for cancer chemotherapy. The concentrations required, however, were ≥ 1 μ M, much higher than found clinically with maximal doses of the cariprazine, a drug that also has active metabolites that accumulate at even higher levels (Nakamura et al., 2016). These facts suggest that repurposing of the parent molecule

might be problematic, and that the reported actions might not be via D₂ or D₃ receptors.

Critical Interpretation and Future Directions

As the literature currently stands, evidence is suggestive, but by no means conclusive, of an anticancer role for D₂R antagonists. Correlative studies of patients with schizophrenia and PD, case studies of cancer patients under concomitant antipsychotic therapy, and repeated hits by unbiased screens support the notion that D₂R may have a significant role in cancer development and may be a reasonable therapeutic target. Also, D₂R antagonists of varying chemotypes have anticancer activity both in vitro and in vivo, where they induce apoptosis, autophagic cell death, and cell cycle arrest (Fig. 1). In some studies, they also induce CSC differentiation and/or disrupt cholesterol trafficking and synthesis. Such effects are favorable for anticancer therapies, especially since these compounds are modestly selective for cancer cells over normal cell type controls of various lineages.

Yet, although these compounds have effects and can affect many signaling pathways (Figure 3), the role of the D₂R itself is still unclear. One major factor is that invariably the concentrations required to induce cytotoxicity are many orders of magnitude higher than the K_D for this receptor. At these concentrations, this class of drug has many off-target actions. As approved drugs, there is a great deal of data regarding pharmacokinetics, pharmacodynamics, and toxicity profiles, which when considered in the light of the modest selectivity in cell culture studies, suggests that it may be difficult to achieve circulating plasma levels sufficient for meaningful anticancer activity (Table 5). Maximal circulating levels are reported as concentrations of parent compound, although some of these compounds would also be present as active or inactive metabolites that may or may not have anticancer activities. Many D₂R antagonists also have profound side effects that included marked increase in serum prolactin, large increases in body weight and metabolic syndrome, neurologic side effects, and potentially fatal cardiac complications like torsades de pointes that results from QT prolongation. Although some of these are quite serious, they may be tolerable in patients with cancers that are unresponsive to other therapies, especially if the side effects are reversible. The question is whether there is an adequate therapeutic window and an adequate degree of efficacy.

Another issue arising from the high concentrations necessary for anticancer effects is that of target determination; it is far from clear that the D₂R is a valid anticancer target on the basis of pharmacological studies alone. Aside from the studies of Li et al. (2014) with GBM, there is little in vitro or in vivo data to suggest that alteration of D₂R levels can affect cell growth, viability, or response to D₂R antagonist treatment. Studies to determine the role of the D₂R will require both understanding of basic principles of pharmacology and the use of orthogonal approaches to decrease the likelihood of erroneous conclusions. Thus, if the D₂R is hypothesized to be the target by which an antipsychotic drug kills or inhibits cancer cell growth, then rigorous evidence must be provided to demonstrate that the receptor is both expressed on the cell type of interest and the principal target that needs to be engaged. Ideally a combination of approaches such as receptor binding assays, Western blot, immunosorting analysis, mRNA

quantification, molecular ablation, and the like are needed to provide a rigorous test of the underlying hypothesis. Without these types of data, assigning activity to a specific target is risky.

As an example, ONC201, a small-molecule inhibitor of TNF-related apoptosis-inducing ligand (TRAIL), reduced proliferation and viability in HCT116 gastric cancer cells (Allen et al., 2016) with antagonism of the D₂R as a major part of its activity. The mechanism was defined further by the suggestion by Leng et al. (2017), who hypothesized a D₅R modulation of the D₂R by direct experiments using the nanomolar affinity D₁/D₅ agonist SKF83959 (Lee et al., 2014a) and the nanomolar affinity D₂-like agonist cabergoline (Newman-Tancredi et al., 2002). They found that ligand concentrations $\geq 5 \mu\text{M}$ or higher were required to cause effects for these drugs. Despite the good selectivity of SKF83959, and to a lesser extent cabergoline, micromolar concentrations will engage many off-target effects as known for SKF83939 (Lee et al., 2014b). On the basis of the pharmacological principles underlying the current analysis, there should be significant skepticism about this proposed D₅R/D₂R mechanism. Shortly thereafter, it was shown that the cytotoxicity of ONC201 was not eliminated by D₂R knockdown or knockout (Kline et al., 2018). Moreover, in a preliminary clinical study against glioblastoma multiforme, ONC201 increased circulating prolactin by only 20% (Arrillaga-Romany et al., 2017), whereas known D₂ antagonists cause multifold increases, inconsistent with effects via the D₂R. Although they had shown that D₂R knockdown or knockout was not the primary mechanism for ONC201 (Kline et al., 2018), these investigators (Prabhu et al., 2019) use associations and correlations of expression data (without any direct assessment of pharmacology or signaling) to elaborate further on the D₅R-D₂R modulation suggested by Leng et al. (2017). A pharmacological analysis suggests that neither receptor is of primary importance.

In summary, we were attracted to this topic because it seemed like an excellent example of the potential for drug repurposing with a known target (i.e., D₂R) for which dozens of drugs are approved, and for which there are probably thousands of experimental compounds that already exist. If the D₂R is a viable target, such a wealth of compounds and data would be a very fertile field for study. Yet, our attempt at a critical view of the literature has altered our initial opinion, such that we believe it is probable that the actions of D₂R antagonists both in vivo and in vitro will not in most cases involve effects mediated primarily by the D₂ receptor. Indeed, novel phenothiazine derivatives have been shown to have many potential anticancer activities aside from the established activities with respect to calmodulin, dopamine receptors, and other known psychiatrically relevant targets. These include antioxidant ability, inhibition of tubulin polymerization, and inhibition of farnesyl transferase (Prinz et al., 2011; Baciú-Atudosie et al., 2012; Engwa et al., 2016; Ghinet et al., 2016). We recognize how our hypothesis, which runs counter to a voluminous literature, could be interpreted, but we believe it would be useful if this generates controversy that leads to hypothesis-driven studies using orthogonal approaches and varying structural series of D₂R antagonists. Such rigorous pharmacological evidence could help clarify many of the intrinsic issues. Whether our supposition is correct or not, the field will benefit from a clear resolution of these questions, and

the knowledge might impact on the development of new therapeutic paradigms.

Acknowledgments

This work was funded by the Penn State Cancer Institute, the Pritchard Distinguished Graduate Fellowship, and the National Cancer Institute (T32CA060395-21A1). The authors declare no conflicts of interest in this work.

Authorship Contributions

Performed data analysis: Weissenrieder, Mailman.

Wrote or contributed to the writing of the manuscript: Weissenrieder, Mailman, Neighbors, Hohl.

Note Added in Proof—Some references and footnote were accidentally not included in Table 3 in the Fast Forward version published April 18, 2019. Table 3 has now been corrected.

References

- Akiyama S, Shiraiishi N, Kuratomi Y, Nakagawa M, and Kuwano M (1986) Circumvention of multiple-drug resistance in human cancer cells by thioridazine, trifluoperazine, and chlorpromazine. *J Natl Cancer Inst* **76**:839–844.
- Allen JE, Kline CL, Prabhu VV, Wagner J, Ishizawa J, Madhukar N, Lev A, Baumeister M, Zhou L, Lulla A, et al. (2016) Discovery and clinical introduction of first-in-class imipridone ONC201. *Oncotarget* **7**:74380–74392.
- Arrillaga-Romany I, Chi AS, Allen JE, Oster W, Wen PY, and Batchelor TT (2017) A phase 2 study of the first imipridone ONC201, a selective DRD2 antagonist for oncology, administered every three weeks in recurrent glioblastoma. *Oncotarget* **8**:79298–79304.
- Baciú-Atudosie L, Ghinet A, Farce A, Dubois J, Beleil D, and Bicu E (2012) Synthesis and biological evaluation of new phenothiazine derivatives bearing a pyrazole unit as protein farnesyltransferase inhibitors. *Bioorg Med Chem Lett* **22**:6896–6902.
- Barak Y, Achiron A, Mandel M, Mirecki I, and Aizenberg D (2005) Reduced cancer incidence among patients with schizophrenia. *Cancer* **104**:2817–2821.
- Beaulieu JM, Gainetdinov RR, and Caron MG (2007) The Akt-GSK-3 signaling cascade in the actions of dopamine. *Trends Pharmacol Sci* **28**:166–172.
- Beaulieu JM, Sotnikova TD, Yao WD, Kockeritz L, Woodgett JR, Gainetdinov RR, and Caron MG (2004) Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc Natl Acad Sci USA* **101**:5099–5104.
- Becker C, Brobert GP, Johansson S, Jick SS, and Meier CR (2010) Cancer risk in association with Parkinson disease: a population-based study. *Parkinsonism Relat Disord* **16**:186–190.
- Besnard J, Ruda GF, Setola V, Abecassis K, Rodriguiz RM, Huang XP, Norval S, Sassano MF, Shin AI, Webster LA, et al. (2012) Automated design of ligands to polypharmacological profiles. *Nature* **492**:215–220.
- Billard W, Ruperto V, Crosby G, Iorio LC, and Barnett A (1984) Characterization of the binding of 3H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. *Life Sci* **35**:1885–1893.
- Boyd KN and Mailman RB (2012) Dopamine receptor signaling and current and future antipsychotic drugs. *Handb Exp Pharmacol*:53–86.
- Brami-Cherrier K, Valjent E, Garcia M, Pages C, Hipkind RA, and Caboche J (2002) Dopamine induces a PI3-kinase-independent activation of Akt in striatal neurons: a new route to cAMP response element-binding protein phosphorylation. *J Neurosci* **22**:8911–8921.
- Bunzow JR, Van Tol HH, Grandy DK, Albert P, Salon J, Christie M, Machida CA, Neve KA, and Civelli O (1988) Cloning and expression of a rat D2 dopamine receptor cDNA. *Nature* **336**:783–787.
- Burstein ES, Ma J, Wong S, Gao Y, Pham E, Knapp AE, Nash NR, Olsson R, Davis RE, Hacksell U, et al. (2005) Intrinsic efficacy of antipsychotics at human D2, D3, and D4 dopamine receptors: identification of the clozapine metabolite N-desmethyloclozapine as a D2/D3 partial agonist. *J Pharmacol Exp Ther* **315**:1278–1287.
- Burt DR, Creese I, and Snyder SH (1976) Properties of [3H]haloperidol and [3H]-dopamine binding associated with dopamine receptors in calf brain membranes. *Mol Pharmacol* **12**:800–812.
- Byun HJ, Lee JH, Kim BR, Kang S, Dong SM, Park MS, Lee SH, Park SH, and Rho SB (2012) Anti-angiogenic effects of thioridazine involving the FAK-mTOR pathway. *Microvasc Res* **84**:227–234.
- Canfrán-Duque A, Casado ME, Pastor O, Sánchez-Wandelmer J, de la Peña G, Lerma M, Mariscal P, Bracher F, Lasunción MA, and Busto R (2013) Atypical antipsychotics alter cholesterol and fatty acid metabolism in vitro. *J Lipid Res* **54**:310–324.
- Carlsson A and Lindqvist M (1963) Effect of chlorpromazine and haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacol Toxicol (Copenh)* **20**:140–144.
- Carlsson A, Lindqvist M, Magnusson T, and Waldeck B (1958) On the presence of 3-hydroxytyramine in brain. *Science* **127**:471.
- Chauvet N, Romanò N, Lafont C, Guillou A, Galibert E, Bonnefont X, Le Tissier P, Fedele M, Fusco A, Mollard P, et al. (2017) Complementary actions of dopamine D2 receptor agonist and anti-vegfr therapy on tumoral vessel normalization in a transgenic mouse model. *Int J Cancer* **140**:2150–2161.
- Cheng HW, Liang YH, Kuo YL, Chuu CP, Lin CY, Lee MH, Wu AT, Yeh CT, Chen EI, Whang-Peng J, et al. (2015) Identification of thioridazine, an antipsychotic drug, as

- an antiangiogenesis and anticancer stem cell agent using public gene expression data. *Cell Death Dis* 6:e1753.
- Cherubini E, Esposito MC, Scozzi D, Terzo F, Osman GA, Mariotti S, Mancini R, Bruno P, and Ricci A (2016) Genetic polymorphism of CHRM2 in COPD: clinical significance and therapeutic implications. *J Cell Physiol* 231:1745–1751.
- Chetty M, Gouws E, Miller R, and Moodley SV (1999) The use of a side effect as a qualitative indicator of plasma chlorpromazine levels. *Eur Neuropsychopharmacol* 9:77–82.
- Chio CL, Hess GF, Graham RS, and Huff RM (1990) A second molecular form of D2 dopamine receptor in rat and bovine caudate nucleus. *Nature* 343:266–269.
- Choi AR, Kim JH, and Yoon S (2014) Thioridazine specifically sensitizes drug-resistant cancer cells through highly increase in apoptosis and P-gp inhibition. *Tumour Biol* 35:9831–9838.
- Choi JH, Yang YR, Lee SK, Kim SH, Kim YH, Cha JY, Oh SW, Ha JR, Ryu SH, and Suh PG (2008) Potential inhibition of PDK1/Akt signaling by phenothiazines suppresses cancer cell proliferation and survival. *Ann NY Acad Sci* 1138:393–403.
- Cortessis V, Ingles S, Millikan R, Diep A, Gatti RA, Richardson L, Thompson WD, Paganini-Hill A, Sparkes RS, and Haile RW (1993) Linkage analysis of DRD2, a marker linked to the ataxia-telangiectasia gene, in 64 families with premenopausal bilateral breast cancer. *Cancer Res* 53:5083–5086.
- Creese I, Burt DR, and Snyder SH (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192:481–483.
- Csatary LK (1972) Chlorpromazines and cancer. *Lancet* 2:338–339.
- Cussac D, Newman-Tancredi A, Sezgin L, and Millan MJ (2000) [3H]S33084: a novel, selective and potent radioligand at cloned, human dopamine D3 receptors. *Naunyn-Schmiedeberg Arch Pharmacol* 361:569–572.
- Daley E, Wilkie D, Loesch A, Hargreaves IP, Kendall DA, Pilkington GJ, and Bates TE (2005) Chlorimipramine: a novel anticancer agent with a mitochondrial target. *Biochem Biophys Res Commun* 328:623–632.
- Dal Toso R, Sommer B, Ewert M, Herb A, Pritchett DB, Bach A, Shivers BD, and Seeburg PH (1989) The dopamine D2 receptor: two molecular forms generated by alternative splicing. *EMBO J* 8:4025–4034.
- Dawkins MJ, Judah JD, and Rees KR (1959) The effect of chlorpromazine on the respiratory chain; cytochrome oxidase. *Biochem J* 72:204–209.
- Delay J and Deniker P (1955) Neuroleptic effects of chlorpromazine in therapeutics of neuropsychiatry. *J Clin Exp Psychopathol* 16:104–112.
- Delay J, Deniker P, Harl, and Grasset A (1952) [N-(dimethylamino-propyl)chlorophenothiazine (4560 RP) therapy of confusional states]. *Ann Med Psychol (Paris)* 110:398–403.
- Deslauriers J, Lefrançois M, Larouche A, Sarret P, and Grignon S (2011) Antipsychotic-induced DRD2 upregulation and its prevention by α -lipoic acid in SH-SY5Y neuroblastoma cells. *Synapse* 65:321–331.
- Dolma S, Selvadurai HJ, Lan X, Lee L, Kushida M, Voisin V, Whetstone H, So M, Aviv T, Park N, et al. (2016) Inhibition of dopamine receptor D4 impedes autophagic flux, proliferation, and survival of glioblastoma stem cells. *Cancer Cell* 29:859–873.
- Driscoll JS, Melnick NR, Quinn FR, Lomax N, Davignon JP, Ing R, Abott BJ, Congleton G, and Dudeck L (1978) Psychotropic drugs as potential antitumor agents: a selective screening study. *Cancer Treat Rep* 62:45–74.
- Driver JA, Logroscino G, Buring JE, Gaziano JM, and Kurth T (2007) A prospective cohort study of cancer incidence following the diagnosis of Parkinson's disease. *Cancer Epidemiol Biomarkers Prev* 16:1260–1265.
- Eaton WW, Mortensen PB, Herrman H, Freeman H, Bilker W, Burgess P, and Wooff K (1992) Long-term course of hospitalization for schizophrenia: part I. Risk for rehospitalization. *Schizophr Bull* 18:217–228.
- Eicke WJ (1973) [Favorable course in carcinoma patients due to additional phenothiazine therapy]. *Med Klin* 68:1015–1018.
- Engwa GA, Ayuk EL, Igbojekwe BU, and Unaegbu M (2016) Potential antioxidant activity of new tetracyclic and pentacyclic nonlinear phenothiazine derivatives. *Biochem Res Int* 2016:9896575.
- Farooqui T, Brooks K, Harrold MW, Miller DD, Wallace LJ, and Uretsky NJ (1994) Interaction of permanently charged metoclopramide analogs with D-2 dopamine receptors. *Gen Pharmacol* 25:1577–1584.
- Fernø J, Skrede S, Vik-Mo AO, Håvik B, and Steen VM (2006) Drug-induced activation of SREBP-controlled lipogenic gene expression in CNS-related cell lines: marked differences between various antipsychotic drugs. *BMC Neurosci* 7:69.
- Froemming JS, Lam YW, Jann MW, and Davis CM (1989) Pharmacokinetics of haloperidol. *Clin Pharmacokinet* 17:396–423.
- Garau L, Govoni S, Stefanini E, Trabucchi M, and Spano PF (1978) Dopamine receptors: pharmacological and anatomical evidences indicate that two distinct dopamine receptor populations are present in rat striatum. *Life Sci* 23:1745–1750.
- Ghinat A, Moise IM, Rigo B, Homerin G, Farce A, Dubois J, and Bicu E (2016) Studies on phenothiazines: new microtubule-interacting compounds with phenothiazine A-ring as potent antineoplastic agents. *Bioorg Med Chem* 24:2307–2317.
- Gil-Ad I, Shtaf B, Levkovitz Y, Dayag M, Zeldich E, and Weizman A (2004) Characterization of phenothiazine-induced apoptosis in neuroblastoma and glioma cell lines: clinical relevance and possible application for brain-derived tumors. *J Mol Neurosci* 22:189–198.
- Giros B, Sokoloff P, Martres MP, Riou JF, Emorine LJ, and Schwartz JC (1989) Alternative splicing directs the expression of two D2 dopamine receptor isoforms. *Nature* 342:923–926.
- Grandy DK, Marchionni MA, Makam H, Stofko RE, Alfano M, Frothingham L, Fischer JB, Burke-Howie KJ, Bunzow JR, Server AC, et al. (1989) Cloning of the cDNA and gene for a human D2 dopamine receptor. *Proc Natl Acad Sci USA* 86:9762–9766.
- Guittion C, Abbar M, Kinowski JM, Chabrand P, and Bressolle F (1998) Multiple-dose pharmacokinetics of clozapine in patients with chronic schizophrenia. *J Clin Psychopharmacol* 18:470–476.
- Gulbinat W, Dupont A, Jablensky A, Jensen OM, Marsella A, Nakane Y, and Sartorius N (1992) Cancer incidence of schizophrenic patients. Results of record linkage studies in three countries. *Br J Psychiatry Suppl* Oct.:75–83.
- Gutierrez A, Pan L, Groen RW, Baleydiere F, Kentsis A, Marineau J, Greblunaite R, Kozakewich E, Reed C, Plumbo F, et al. (2014) Phenothiazines induce PP2A-mediated apoptosis in T cell acute lymphoblastic leukemia. *J Clin Invest* 124:644–655.
- Hamaue N, Yamazaki N, Terado M, Minami M, Ohno K, Ide H, Ogata A, Honma S, and Tashiro K (2000) Urinary isatin concentrations in patients with Parkinson's disease determined by a newly developed HPLC-UV method. *Res Commun Mol Pathol Pharmacol* 108:63–73.
- Hercbergs A (1988) Thioridazine: a radiation enhancer in advanced cervical cancer? *Lancet* 2:737.
- Hirsch L, Yang J, Bresee L, Jette N, Patten S, and Pringsheim T (2017) Second-generation antipsychotics and metabolic side effects: a systematic review of population-based studies. *Drug Saf* 40:771–781.
- Hoepfner LH, Wang Y, Sharma A, Javed N, Van Keulen VP, Wang E, Yang P, Roden AC, Peikert T, Molina JR, et al. (2015) Dopamine D2 receptor agonists inhibit lung cancer progression by reducing angiogenesis and tumor infiltrating myeloid derived suppressor cells. *Mol Oncol* 9:270–281.
- Hoffman RP (2017) The complex inter-relationship between diabetes and schizophrenia. *Curr Diabetes Rev* 13:528–532.
- Hussein N, Amawi H, Karthikeyan C, Hall FS, Mittal R, Trivedi P, Ashby CR Jr, and Tiwari AK (2017) The dopamine D3 receptor antagonists PG01037, NGB2904, SB277011A, and U99194 reverse ABCG2 transporter-mediated drug resistance in cancer cell lines. *Cancer Lett* 396:167–180.
- Hussein N, Ashby CR Jr, Amawi H, Nyinawabera A, Vij A, Khare VM, Karthikeyan C, and Tiwari AK (2018) Cariprazine, a dopamine D2/D3 receptor partial agonist, modulates ABCG2-mediated multidrug resistance in cancer. *Cancers (Basel)* 10.
- Iishi H, Baba M, Tatsuta M, Okuda S, and Taniguchi H (1992) Enhancement of dopaminergic agonist bromocriptine of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. *Br J Cancer* 65:351–354.
- Ika S, Suzuki T, Mimura M, and Uchida H (2016) Plasma levels and estimated dopamine D2 receptor occupancy of long-acting injectable risperidone during maintenance treatment of schizophrenia: a 3-year follow-up study. *Psychopharmacology (Berl)* 233:4003–4010.
- Jacobs H and Franks S (1975) Letter: prolactin studies, pituitary tumour, and reproductive function. *BMJ* 2:141–142.
- Jagetai GC and Ganapathi NG (1991) Treatment of mice with a herbal preparation (Liv. 52) reduces the frequency of radiation-induced chromosome damage in bone marrow. *Mutat Res* 253:123–126.
- Jandaghi P, Najafabadi HS, Bauer AS, Papadakis AI, Fassin M, Hall A, Monast A, von Knebel Doeberitz M, Neoptolemos JP, Costello E, et al. (2016) Expression of DRD2 is increased in human pancreatic ductal adenocarcinoma and inhibitors slow tumor growth in mice. *Gastroenterology* 151:1218–1231.
- Jansson B and Jankovic J (1985) Low cancer rates among patients with Parkinson's disease. *Ann Neurol* 17:505–509.
- Jennings A, Tyurikova O, Bard L, Zheng K, Semyanov A, Henneberger C, and Rusakov DA (2017) Dopamine elevates and lowers astroglial Ca2+ through distinct pathways depending on local synaptic circuitry. *Glia* 65:447–459.
- Jespersen CG, Nørgaard M, and Borre M (2016) Parkinson's disease and risk of prostate cancer: a Danish population-based case-control study, 1995–2010. *Cancer Epidemiol* 45:157–161.
- Kanakis G, Grmelius L, Spathis A, Tringidou R, Rassidakis GZ, Öberg K, Kaltsas G, and Tsolakis AV (2015) Expression of somatostatin receptors 1-5 and dopamine receptor 2 in lung carcinoids: implications for a therapeutic role. *Neuroendocrinology* 101:211–222.
- Kang S, Dong SM, Kim BR, Park MS, Trink B, Byun HJ, and Rho SB (2012) Thioridazine induces apoptosis by targeting the PI3K/Akt/mTOR pathway in cervical and endometrial cancer cells. *Apoptosis* 17:989–997.
- Karpel-Massler G, Kast RE, Westhoff MA, Dwucet A, Welscher N, Nonnenmacher L, Hlavac M, Siegelin MD, Wirtz CR, Debatin KM, et al. (2015) Olanzapine inhibits proliferation, migration and anchorage-independent growth in human glioblastoma cell lines and enhances temozolomide's antiproliferative effect. *J Neurooncol* 122:21–33.
- Kassahun K, Mattiuz E, Nyhart E Jr, Obermeyer B, Gillespie T, Murphy A, Goodwin RM, Tupper D, Callaghan JT, and Lemberger L (1997) Disposition and bio-transformation of the antipsychotic agent olanzapine in humans. *Drug Metab Dispos* 25:81–93.
- Kau TR, Schroeder F, Ramaswamy S, Wojciechowski CL, Zhao JJ, Roberts TM, Clardy J, Sellers WR, and Silver PA (2003) A chemical genetic screen identifies inhibitors of regulated nuclear export of a Forkhead transcription factor in PTEN-deficient tumor cells. *Cancer Cell* 4:463–476.
- Kebabian JW and Calne DB (1979) Multiple receptors for dopamine. *Nature* 277: 93–96.
- Kerbusch T, Desta Z, Soukhova NV, Thacker D, and Flockhart DA (1997) Sensitive assay for pimozone in human plasma using high-performance liquid chromatography with fluorescence detection: application to pharmacokinetic studies. *J Chromatogr B Biomed Sci Appl* 694:163–168.
- Kihara T, Shimohama S, Sawada H, Honda K, Nakamizo T, Kanki R, Yamashita H, and Akaike A (2002) Protective effect of dopamine D2 agonists in cortical neurons via the phosphatidylinositol 3 kinase cascade. *J Neurosci Res* 70:274–282.
- Kline CLB, Ralf MD, Lulla AR, Wagner JM, Abbosh PH, Dicker DT, Allen JE, and El-Deiry WS (2018) Role of dopamine receptors in the anticancer activity of ONC201. *Neoplasia* 20:80–91.
- Kristiana I, Sharpe LJ, Catts VS, Lutze-Mann LH, and Brown AJ (2010) Antipsychotic drugs upregulate lipogenic gene expression by disrupting intracellular trafficking of lipoprotein-derived cholesterol. *Pharmacogenomics J* 10:396–407.
- Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, Ernsberger P, Jayathilake K, Meltzer HY, and Roth BL (2003) H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology* 28:519–526.
- Lawler CP, Prioleau C, Lewis MM, Mak C, Jiang D, Schetz JA, Gonzalez AM, Sibley DR, and Mailman RB (1999) Interactions of the novel antipsychotic aripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. *Neuropsychopharmacol* 20:612–627.

- Lee SM, Kant A, Blake D, Murthy V, Boyd K, Wyrick SJ, and Mailman RB (2014a) SKF-83959 is not a highly-biased functionally selective D1 dopamine receptor ligand with activity at phospholipase C. *Neuropharmacology* **86**:145–154.
- Lee SM, Yang Y, and Mailman RB (2014b) Dopamine D1 receptor signaling: does Gαq-phospholipase C actually play a role? *J Pharmacol Exp Ther* **351**:9–17.
- Leng ZG, Lin SJ, Wu ZR, Guo YH, Cai L, Shang HB, Tang H, Xue YJ, Lou MQ, Zhao W, et al. (2017) Activation of DRD5 (dopamine receptor D5) inhibits tumor growth by autophagic cell death. *Autophagy* **13**:1404–1419.
- Levkovitz Y, Gil-Ad I, Zeldich E, Dayag M, and Weizman A (2005) Differential induction of apoptosis by antidepressants in glioma and neuroblastoma cell lines: evidence for p-c-Jun, cytochrome c, and caspase-3 involvement. *J Mol Neurosci* **27**:29–42.
- Li J, Zhu S, Kozono D, Ng K, Futalan D, Shen Y, Akers JC, Steed T, Kushwaha D, Schlabach M, et al. (2014) Genome-wide shRNA screen revealed integrated mitogenic signaling between dopamine receptor D2 (DRD2) and epidermal growth factor receptor (EGFR) in glioblastoma. *Oncotarget* **5**:882–893.
- Li L, Miyamoto M, Ebihara Y, Mega S, Takahashi R, Hase R, Kaneko H, Kadoya M, Itoh T, Shichinohe T, et al. (2006) DRD2/DARPP-32 expression correlates with lymph node metastasis and tumor progression in patients with esophageal squamous cell carcinoma. *World J Surg* **30**:1672–1679, discussion 1680–1681.
- Lichtermann D, Ekelund J, Pukkala E, Tanskanen A, and Lönnqvist J (2001) Incidence of cancer among persons with schizophrenia and their relatives. *Arch Gen Psychiatry* **58**:573–578.
- Lu D and Carson DA (2009) Spiperone enhances intracellular calcium level and inhibits the Wnt signaling pathway. *BMC Pharmacol* **9**:13.
- Mailman R, Huang X, and Nichols DE (2001) Parkinson's disease and D1 dopamine receptors. *Curr Opin Investig Drugs* **2**:1582–1591.
- Mailman RB (2007) GPCR functional selectivity has therapeutic impact. *Trends Pharmacol Sci* **28**:390–396.
- Mailman RB and Huang X (2007) Dopamine receptor pharmacology, in *Parkinson's Disease and Related Disorders, Part 1* (Koller WC and Melamed E eds) pp 77–105, Elsevier, New York.
- Mannoury la Cour C, Salles MJ, Pasteau V, and Millan MJ (2011) Signaling pathways leading to phosphorylation of Akt and GSK-3β by activation of cloned human and rat cerebral D₂ and D₃ receptors. *Mol Pharmacol* **79**:91–105.
- Mao M, Yu T, Hu J, and Hu L (2015) Dopamine D2 receptor blocker thioridazine induces cell death in human uterine cervical carcinoma cell line SiHa. *J Obstet Gynaecol Res* **41**:1240–1245.
- Masson M, Spezzatti B, Chapman J, Battisti C, and Baumann N (1992) Calmodulin antagonists chlorpromazine and W-7 inhibit exogenous cholesterol esterification and sphingomyelinase activity in human skin fibroblast cultures. Similarities between drug-induced and Niemann-Pick type C lipidoses. *J Neurosci Res* **31**:84–88.
- Meng Q, Sun X, Wang J, Wang Y, and Wang L (2016) The important application of thioridazine in the endometrial cancer. *Am J Transl Res* **8**:2767–2775.
- Millan MJ, Gobert A, Lejeune F, Newman-Tancredi A, Rivet JM, Auclair A, and Peglion JL (2001) S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. *J Pharmacol Exp Ther* **298**:565–580.
- Min KJ, Seo BR, Bae YC, Yoo YH, and Kwon TK (2014) Antipsychotic agent thioridazine sensitizes renal carcinoma Caki cells to TRAIL-induced apoptosis through reactive oxygen species-mediated inhibition of Akt signaling and downregulation of Mcl-1 and c-FLIP(L). *Cell Death Dis* **5**:e1063.
- Møller H, Møllenkjaer L, McLaughlin JK, and Olsen JH (1995) Occurrence of different cancers in patients with Parkinson's disease. *BMJ* **310**:1500–1501.
- Monsma FJ Jr, Brassard DL, and Sibley DR (1989a) Identification and characterization of D1 and D2 dopamine receptors in cultured neuroblastoma and retinoblastoma clonal cell lines. *Brain Res* **492**:314–324.
- Monsma FJ Jr, McVittie LD, Gerfen CR, Mahan LC, and Sibley DR (1989b) Multiple D2 dopamine receptors produced by alternative RNA splicing. *Nature* **342**:926–929.
- Moreno-Smith M, Lu C, Shahzad MM, Pena GN, Allen JK, Stone RL, Mangala LS, Han HD, Kim HS, Farley D, et al. (2011) Dopamine blocks stress-mediated ovarian carcinoma growth. *Clin Cancer Res* **17**:3649–3659.
- Mortensen PB (1994) The occurrence of cancer in first admitted schizophrenic patients. *Schizophr Res* **12**:185–194.
- Mu J, Xu H, Yang Y, Huang W, Xiao J, Li M, Tan Z, Ding Q, Zhang L, Lu J, et al. (2014) Thioridazine, an antipsychotic drug, elicits potent antitumor effects in gastric cancer. *Oncol Rep* **31**:2107–2114.
- Munk-Jørgensen P and Mortensen PB (1989) Schizophrenia: a 13-year follow-up. Diagnostic and psychopathological aspects. *Acta Psychiatr Scand* **79**:391–399.
- Nagel D, Spranger S, Vincendeau M, Grau M, Raffegierst S, Kloob B, Hlahla D, Neuschwander M, Peter von Kries J, Hadian K, et al. (2012) Pharmacologic inhibition of MALT1 protease by phenothiazines as a therapeutic approach for the treatment of aggressive ABC-DLBCL. *Cancer Cell* **22**:825–837.
- Nakamura T, Kubota T, Iwakaji A, Imada M, Kapás M, and Morio Y (2016) Clinical pharmacology study of cariprazine (MP-214) in patients with schizophrenia (12-week treatment). *Drug Des Devel Ther* **10**:327–338.
- Nelson EA, Walker SR, Weisberg E, Bar-Natan M, Barrett R, Gashin LB, Terrell S, Klitgaard JL, Santo L, Addorio MR, et al. (2011) The STAT5 inhibitor pimozone decreases survival of chronic myelogenous leukemia cells resistant to kinase inhibitors. *Blood* **117**:3421–3429.
- Neumeyer JL, Kula NS, Bergman J, and Baldessarini RJ (2003) Receptor affinities of dopamine D1 receptor-selective novel phenylbenzazepines. *Eur J Pharmacol* **474**:137–140.
- Neve KA and Neve RL (1997) *The Dopamine Receptors*, Humana Press, Totowa, NJ.
- Newman-Tancredi A, Cussac D, Audinot V, Nicolas JP, De Ceuninck F, Boutin JA, and Millan MJ (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. II. Agonist and antagonist properties at subtypes of dopamine D(2)-like receptor and alpha(1)/alpha(2)-adrenoceptor. *J Pharmacol Exp Ther* **303**:805–814.
- Osterman E (1961) [Has chlorpromazine a cytostatic effect? A few reflections in connection with a case and the modern literature]. *Nord Psykiatr Tidsskr* **15**:154–159.
- Park MS, Dong SM, Kim BR, Seo SH, Kang S, Lee EJ, Lee SH, and Rho SB (2014) Thioridazine inhibits angiogenesis and tumor growth by targeting the VEGFR-2/P13K/mTOR pathway in ovarian cancer xenografts. *Oncotarget* **5**:4929–4934.
- Park SH, Chung YM, Ma J, Yang Q, Berek JS, and Hu MC (2016) Pharmacological activation of FOXO3 suppresses triple-negative breast cancer in vitro and in vivo. *Oncotarget* **7**:42110–42125.
- Pornour M, Ahangari G, Hejazi SH, Ahmadvani HR, and Akbari ME (2014) Dopamine receptor gene (DRD1-DRD5) expression changes as stress factors associated with breast cancer. *Asian Pac J Cancer Prev* **15**:10339–10343.
- Prabhu VV, Madhukar NS, Gilvary C, Kline CLB, Oster S, El-Deiry WS, Elemento O, Doherty F, VanEngelenburg A, Durrant J, et al. (2019) Dopamine receptor D5 is a modulator of tumor response to dopamine receptor D2 antagonism. *Clin Cancer Res* **25**:2305–2313.
- Prinz H, Chamasmani B, Vogel K, Böhm KJ, Aicher B, Gerlach M, Günther EG, Amon P, Ivanov I, and Müller K (2011) N-benzoylated phenothiazines and phenothiazines: synthesis, antiproliferative activity, and inhibition of tubulin polymerization. *J Med Chem* **54**:4247–4263.
- Qi L and Ding Y (2013) Potential antitumor mechanisms of phenothiazine drugs. *Sci China Life Sci* **56**:1020–1027.
- Ranjana A, Gupta P, and Srivastava SK (2016) Penfluridol: an antipsychotic agent suppresses metastatic tumor growth in triple-negative breast cancer by inhibiting integrin signaling axis. *Cancer Res* **76**:877–890.
- Ranjana A and Srivastava SK (2016) Penfluridol suppresses pancreatic tumor growth by autophagy-mediated apoptosis. *Sci Rep* **6**:26165.
- Rho SB, Kim BR, and Kang S (2011) A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway in ovarian cancer cells. *Gynecol Oncol* **120**:121–127.
- Roth BL, Tandra S, Burgess LH, Sibley DR, and Meltzer HY (1995) D4 dopamine receptor binding affinity does not distinguish between typical and atypical antipsychotic drugs. *Psychopharmacology (Berl)* **120**:365–368.
- Sachles E, Riuseno RM, Laronde S, Shapovalova Z, Lee JH, Russell J, Malig M, McNicol JD, Fiebig-Comyn A, Graham M, et al. (2012) Identification of drugs including a dopamine receptor antagonist that selectively target cancer stem cells. *Cell* **149**:1284–1297.
- Sánchez-Wandelmer J, Dávalos A, de la Peña G, Cano S, Giera M, Canfrán-Duque A, Bracher F, Martín-Hidalgo A, Fernández-Hernando C, Lasunción MA, et al. (2010) Haloperidol disrupts lipid rafts and impairs insulin signaling in SH-SY5Y cells. *Neuroscience* **167**:143–153.
- Schalop L and Allen JE (2016) The role of DRD2 in cancer, in *Drug Discovery and Development*, R & D Magazine, Rockaway, NJ.
- Schmidt K, Lucignani G, Moresco RM, Rizzo G, Gilardi MC, Messa C, Colombo F, Fazio F, and Sokoloff L (1992) Errors introduced by tissue heterogeneity in estimation of local cerebral glucose utilization with current kinetic models of the [18F]fluorodeoxyglucose method. *J Cereb Blood Flow Metab* **12**:823–834.
- Seeman P (1995) Therapeutic receptor-blocking concentrations of neuroleptics. *Int Clin Psychopharmacol* **10** (Suppl 3):5–13.
- Seeman P, Corbett R, and Van Tol HH (1997) Atypical neuroleptics have low affinity for dopamine D2 receptors or are selective for D4 receptors. *Neuropsychopharmacology* **16**:93–110, discussion 111–135.
- Seeman P, Lee T, Chau-Wong M, and Wong K (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature* **261**:717–719.
- Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu LX, Sibley DR, Roth BL, and Mailman R (2003) Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology* **28**:1400–1411.
- Shchors K, Massaras A, and Hanahan D (2015) Dual targeting of the autophagic regulatory circuitry in gliomas with repurposed drugs elicits cell-lethal autophagy and therapeutic benefit. *Cancer Cell* **28**:456–471.
- Shin JH, Park SJ, Kim ES, Jo YK, Hong J, and Cho DH (2012) Sertindole, a potent antagonist at dopamine D₂ receptors, induces autophagy by increasing reactive oxygen species in SH-SY5Y neuroblastoma cells. *Biol Pharm Bull* **35**:1069–1075.
- Shin SY, Lee KS, Choi YK, Lim HJ, Lee HG, Lim Y, and Lee YH (2013) The antipsychotic agent chlorpromazine induces autophagic cell death by inhibiting the Akt/mTOR pathway in human U-87MG glioma cells. *Carcinogenesis* **34**:2080–2089.
- Sigwald J and Bouttier D (1953) [Utilization of the neuroleptic properties of chloro-3-(dimethylamino-3'-propyl)-10-phenothiazine hydrochloride (R.P. 4560) in neuropsychiatric therapy]. *Presse Med* **61**:607–609.
- Smith RC, Baumgartner R, Ravichandran GK, Shvartsburd A, Schoolar JC, Allen P, and Johnson R (1984) Plasma and red cell levels of thioridazine and clinical response in schizophrenia. *Psychiatry Res* **12**:287–296.
- Sotnikova TD, Beaulieu JM, Barak LS, Wetsel WC, Caron MG, and Gainetdinov RR (2005) Dopamine-independent locomotor actions of amphetamines in a novel acute mouse model of Parkinson disease. *PLoS Biol* **3**:e271.
- Stojanovic T, Orlova M, Sialana FJ, Höger H, Stuchlik S, Milenkovic I, Aradska J, and Lubec G (2017) Validation of dopamine receptor DRD1 and DRD2 antibodies using receptor deficient mice. *Amino Acids* **49**:1101–1109.
- Strobl JS, Kirkwood KL, Lantz TK, Lewine MA, Peterson VA, and Worley JF III (1990) Inhibition of human breast cancer cell proliferation in tissue culture by the neuroleptic agents pimozone and thioridazine. *Cancer Res* **50**:5399–5405.
- Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G, George SR, Torchia J, Van Tol HH, and Niznik HB (1991) Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature* **350**:614–619.
- Sutton LP and Rushlow WJ (2012) The dopamine D2 receptor regulates Akt and GSK-3 via Dvl-3. *Int J Neuropsychopharmacol* **15**:965–979.
- Suzuki S, Okada M, Kuramoto K, Takeda H, Sakaki H, Watarai H, Sanomachi T, Seino S, Yoshioka T, and Kitahara C (2016) Aripiprazole, an antipsychotic and

- partial dopamine agonist, inhibits cancer stem cells and reverses chemoresistance. *Anticancer Res* **36**:5153–5161.
- Tang L, Todd RD, Heller A, and O'Malley KL (1994) Pharmacological and functional characterization of D2, D3 and D4 dopamine receptors in fibroblast and dopaminergic cell lines. *J Pharmacol Exper Ther* **268**:495–502.
- Taylor JR, Lawrence MS, Redmond DE Jr, Elsworth JD, Roth RH, Nichols DE, and Mailman RB (1991) Dihydropyridine, a full dopamine D1 agonist, reduces MPTP-induced parkinsonism in monkeys. *Eur J Pharmacol* **199**:389–391.
- Toll L, Berzetei-Gurske IP, Polgar WE, Brandt SR, Adapa ID, Rodriguez L, Schwartz RW, Haggart D, O'Brien A, White A, et al. (1998) Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. *NIDA Res Monogr* **178**:440–466.
- Van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, and Civelli O (1991) Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature* **350**:610–614.
- Vik-Mo AO, Fernø J, Skrede S, and Steen VM (2009) Psychotropic drugs up-regulate the expression of cholesterol transport proteins including ApoE in cultured human CNS- and liver cells. *BMC Pharmacol* **9**:10.
- Villa GR, Hulce JJ, Zanca C, Bi J, Ikegami S, Cahill GL, Gu Y, Lum KM, Masui K, Yang H, et al. (2016) An LXR-cholesterol Axis creates a metabolic Co-dependency for brain cancers. *Cancer Cell* **30**:683–693.
- Wang V, Chao TH, Hsieh CC, Lin CC, and Kao CH (2015) Cancer risks among the users of ergot-derived dopamine agonists for Parkinson's disease, a nationwide population-based survey. *Parkinsonism Relat Disord* **21**:18–22.
- Wolfe SE and Morris SJ (1999) Dopamine D2 receptor isoforms expressed in AtT20 cells differentially couple to G proteins to acutely inhibit high voltage-activated calcium channels. *J Neurochem* **73**:2375–2382.
- Wu BJ, Lin CH, Tseng HF, Liu WM, Chen WC, Huang LS, Sun HJ, Chiang SK, and Lee SM (2013) Validation of the Taiwanese Mandarin version of the Personal and Social Performance scale in a sample of 655 stable schizophrenic patients. *Schizophr Res* **146**:34–39.
- Wu CH, Bai LY, Tsai MH, Chu PC, Chiu CF, Chen MY, Chiu SJ, Chiang JH, and Weng JR (2016) Pharmacological exploitation of the phenothiazine antipsychotics to develop novel antitumor agents-A drug repurposing strategy. *Sci Rep* **6**:27540.
- Yan Z, Feng J, Fienberg AA, and Greengard P (1999) D(2) dopamine receptors induce mitogen-activated protein kinase and cAMP response element-binding protein phosphorylation in neurons. *Proc Natl Acad Sci USA* **96**:11607–11612.
- Yeh CT, Wu AT, Chang PM, Chen KY, Yang CN, Yang SC, Ho CC, Chen CC, Kuo YL, Lee PY, et al. (2012) Trifluoperazine, an antipsychotic agent, inhibits cancer stem cell growth and overcomes drug resistance of lung cancer. *Am J Respir Crit Care Med* **186**:1180–1188.
- Yin T, He S, Shen G, Ye T, Guo F, and Wang Y (2015) Dopamine receptor antagonist thioridazine inhibits tumor growth in a murine breast cancer model. *Mol Med Rep* **12**:4103–4108.
- Yoon S and Baik JH (2013) Dopamine D2 receptor-mediated epidermal growth factor receptor transactivation through a disintegrin and metalloprotease regulates dopaminergic neuron development via extracellular signal-related kinase activation. *J Biol Chem* **288**:28435–28446.
- Yoon S, Choi MH, Chang MS, and Baik JH (2011) Wnt5a-dopamine D2 receptor interactions regulate dopamine neuron development via extracellular signal-regulated kinase (ERK) activation. *J Biol Chem* **286**:15641–15651.
- Yue H, Huang D, Qin L, Zheng Z, Hua L, Wang G, Huang J, and Huang H (2016) Targeting lung cancer stem cells with antipsychological drug thioridazine. *BioMed Res Int* **2016**:6709828.
- Zhelev Z, Ohba H, Bakalova R, Hadjimitova V, Ishikawa M, Shinohara Y, and Baba Y (2004) Phenothiazines suppress proliferation and induce apoptosis in cultured leukemic cells without any influence on the viability of normal lymphocytes. Phenothiazines and leukemia. *Cancer Chemother Pharmacol* **53**:267–275.
- Zhou W, Chen MK, Yu HT, Zhong ZH, Cai N, Chen GZ, Zhang P, and Chen JJ (2016) The antipsychotic drug pimozide inhibits cell growth in prostate cancer through suppression of STAT3 activation. *Int J Oncol* **48**:322–328.
- Zong D, Zielinska-Chomej K, Juntti T, Mörk B, Lewensohn R, Hååg P, and Viktorsson K (2014) Harnessing the lysosome-dependent antitumor activity of phenothiazines in human small cell lung cancer. *Cell Death Dis* **5**:e1111.

Address correspondence to: Dr. Raymond J. Hohl, Penn State Cancer Institute, Penn State College of Medicine, 500 University Drive, Mail Code CH72, Hershey, PA 17033-0850. E-mail: rhohl@pennstatehealth.psu.edu
